THE EFFECT OF THEOPHYLLINE ON THE RESPIRATORY AND QUADRICEPS FEMORIS MUSCLES IN MAN.

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ABSTRACT

Twitch tension in fresh and fatigued skeletal muscle strips is increased by methylxanthines. In animals, a significant inotropic effect occurs only at theophylline concentrations which are above the therapeutic range in man, while changes at therapeutic concentrations of theophylline in normal subjects and in patients are inconsistent. It has been suggested that patients with respiratory muscle weakness or who are at risk of fatigue may benefit from enhanced respiratory muscle contractility with dimethylxanthines. The magnitude of the effect on respiratory muscle contractility at therapeutic concentrations, and the effect on maximal contractions are not known.

The action of dimethylxanthines on the contractility of respiratory and quadriceps femoris muscle in normal subjects and in patients at risk of respiratory muscle fatigue was assessed using accepted techniques.

The acute effects of aminophylline were investigated in two open studies. In four normal volunteers, twitch tension was not enhanced. However, in a quadriplegic patient, paced transdiaphragmatic pressure significantly increased by 12.6%.

The effect of chronic theophylline was studied in three double-blind randomised, placebo-controlled trials. In the first study of six normal subjects, theophylline had no effect on quadriceps strength, on fatigue-development, or on the shape of the fresh or fatigued frequency-force curve. However, a small treatment difference (1-2%) was noted in low frequency

(20Hz) contractions before and after fatigue.

In five normals, sniff transdiaphragmatic pressure significantly increased by 4.1%, but there were no changes in global respiratory or quadriceps muscle strength.

In 10 patients with chronic obstructive pulmonary disease there were increases in maximal inspiratory mouth pressures at residual volume and functional residual capacity of 11.4% and 18% respectively. Pulmonary function, six minute walk, breathlessness scores, maximal expiratory mouth pressures, and quadriceps strength did not differ between treatment periods.

In all studies, theophylline levels were within the therapeutic range.

Despite the small numbers, a positive inotropic action of theophylline was identified. The improvement in muscle contractility varied between studies: approximately 5% in normal subjects, and 15% in patients. The variable response may be related to the presence or absence of skeletal muscle weakness, fatigue, and factors which potentiate the development of fatigue, such as hypoxia, hyperinflation and raised inspiratory muscle work loads.

DECLARATION

None of the work referred to in this thesis has been submitted in support of an application for another thesis or degree at this or any other University. To the best of my knowledge and belief, this thesis contains no material previously written or published by another person, except where due reference is made in the text of the thesis.

I am willing for the thesis to be available for photocopying and loan if it is accepted for the award of the degree.

Signed.. Date ..17/6/93.....

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ABBREVIATIONS

Abbreviations are listed in alphabetical order.

Abdo - Abdominal.

AP - Anteroposterior.

ATP - Adenosine triphosphate.

AWR - Airway resistance.

cmsH_2O - centimetres of water.

COPD - Chronic obstructive pulmonary disease.

EMG - Electromyogram.

Edi - Electrical activity of the diaphragm.

FEV_1 - Forced expiratory volume in one second.

FFC - Frequency-force curve.

FRC - Functional residual capacity.

FVC - Forced vital capacity.

Hz - Hertz.

Kgs - Kilograms.

l - Litres.

L_o - Resting muscle length.

Lt - Left

m - Metres.

min - Minutes.

mcg - Microgram

mls - Millilitres.

mms - Millimetres.

MRR - Maximal relaxation rate.

usec - Microseconds.

msec - Milliseconds.

MVC - Maximal voluntary contraction.

Pab - Abdominal pressure.

pCO_2 - Partial pressure of carbon dioxide in arterial blood.

pH - Concentration of hydrogen ions in arterial blood.

pO_2 - Partial pressure of oxygen in arterial blood.

Pdi - Transdiaphragmatic pressure.

PEFR - Peak expiratory flow rate.

Pg - Gastric pressure.

Pm - Mouth pressure.

Poes - Oesophageal pressure.

Ppl - Pleural pressure.

RC - Rib cage.

Rt - Right.

RV - Residual volume.

sec - Seconds.

TB - Tidal breathing.

TLC - Total lung capacity.

T_{lim} - the time taken for the diaphragm to fatigue.

TT_{di} - the tension-time index of the diaphragm.

VAS - Visual analogue scale.

VC - Vital capacity.

Vol - Volume.

CHAPTER 1

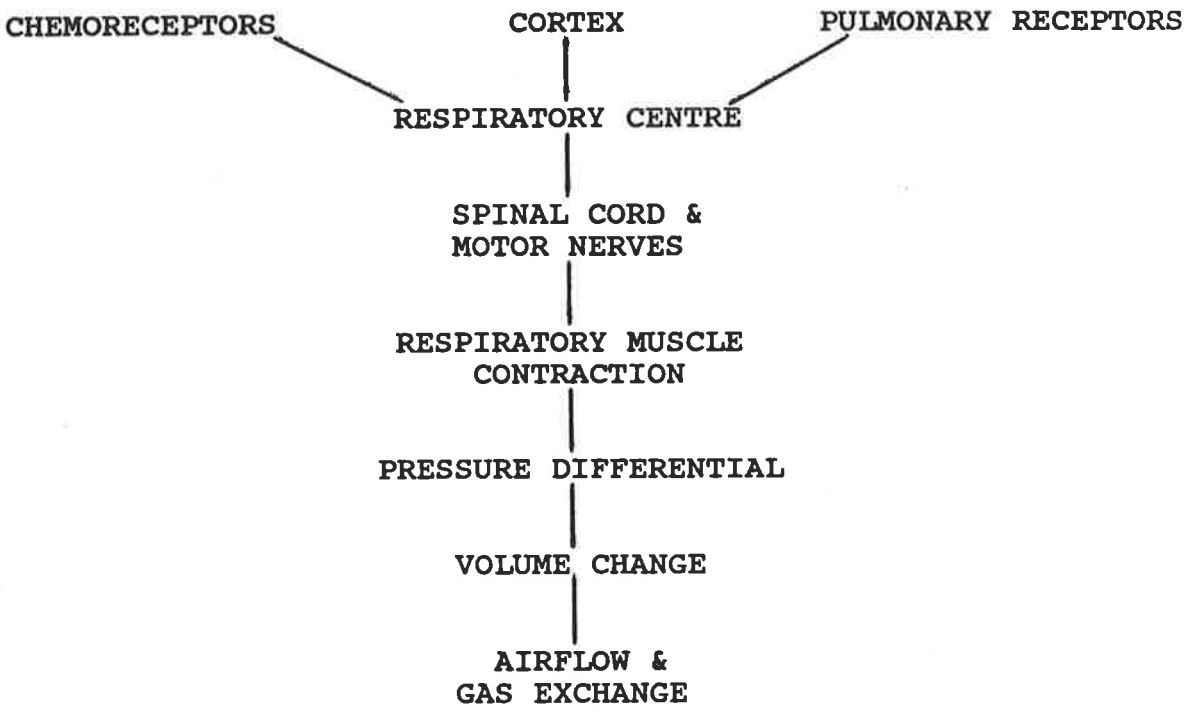
INTRODUCTION, HISTORICAL REVIEW AND OBJECTIVES

SECTION 1 - INTRODUCTION

Ventilation is a complex process dependent on the cyclical firing of central respiratory neurones and transmission of impulses via nerves to the respiratory muscles, which contract causing expansion of the thorax. The resultant pressure differential between the atmosphere and thoracic cavity produces airflow into the lungs (Figure 1.1). Exchange of oxygen and carbon dioxide then occurs between the alveoli and pulmonary capillaries. Abnormality of any of these processes results in impairment of ventilation. In particular, weakness or reduction in optimal function of the respiratory muscles may lead to hypoxia and hypercapnia through poor chest expansion and consequent inadequate passage of air into and out of the lungs.

Chronic bronchitis and emphysema are common disorders causing dyspnoea, hypoxia and hypercapnia, thought to be due, in part, to inadequate respiratory muscle function. Roussos and Macklem (1977) and Bellemare and Grassino (1982a) have shown that the greater the respiratory muscle work, measured as the product of the duration and degree of muscle contraction, the less sustainable it becomes. If the respiratory muscles are required to contract beyond a certain duration and proportion of maximum pressure generation they may eventually lose their capacity to generate the required force (Bellemare & Grassino 1982a). They then have been said to have fatigued. During normal ventilation the respiratory system operates below this critical level.

FIGURE 1.1



The sequence of events producing ventilation. Respiratory effort is initiated by the respiratory centre in the medulla oblongata, and modified by input from higher centres and peripheral receptors. The result is contraction of the respiratory muscles, change in lung volume, and finally gas exchange.

However the combination of reduced strength of the respiratory muscles due to hyperinflation of the chest (Dodd et al 1984, Mier et al 1985a), reduced muscle bulk and hypercapnia (Rochester & Braun 1985) and the requirement to generate high pressures to overcome the resistive and elastic loads (Martin et al 1983), means that in chronic bronchitis and emphysema this critical point may be reached.

Dimethylxanthines are thought to be useful in the management of patients with airflow obstruction. Traditionally, this has been considered to be due to bronchodilatation. However, the genuine benefit to airflow appears to be small (Rossing et al 1980) and recently it has been postulated that some of their beneficial effect may be due to increased respiratory muscle contraction with consequent improvement in ventilation and prevention or reduction of fatigue.

<u>In vitro</u> studies of animal and human skeletal muscle strips have found enhanced twitch tension and protection from fatigue with methylxanthines (Huidobro & Amenbar 1945, Sandow & Brust 1966, Kentera & Varagic 1975, Howell et al 1981, Jones et al 1982). However Jones et al (1982) concluded from their animal work that the amount of theophylline needed for such effects in man would result in toxic serum theophylline levels. Further work in normal volunteers on adductor pollicis muscle fatigue by Wiles and colleagues (1983), and on diaphragmatic twitch tension by Moxham and co-workers (1985) failed to reveal an effect of aminophylline at therapeutic concentrations. Other workers have demonstrated increased diaphramatic contraction

and prevention of fatigue in normal subjects (Aubier et al 1981a, Supinski et al 1984a). Since the start of this thesis, there has been a report of use of theophylline over several weeks in patients with chronic airways obstruction resulting in improved respiratory muscle force development and reduction in the susceptibility to respiratory muscle fatigue (Murciano et al 1984). Others, however, have failed to corroborate a clinical benefit in terms of reduced breathlessness in such patients who are thought to be most at risk of respiratory muscle fatigue (Eaton et al 1982, Belman et al 1985).

At the time of starting this work (April 1983), there were no studies investigating the action of methylxanthines on global respiratory muscle strength. This is relevant to the potential for methylxanthines to prevent fatigue, as reduced strength and the proportion of maximum to which respiratory muscles contract to maintain ventilation are critical factors in fatigue development. In addition, little work had been done on the action of aminophyllline on the diaphragmatic twitch, which is known to be affected by methylxanthines, nor on the bilateral twitch, more relevant than the unilateral twitch to physiological diaphragm contraction. Other potentially at risk groups, eg. patients with phrenic nerve pacers and wasted diaphragms, had not been assessed to determine the effects of aminophylline.

This work describes a prospective study of the potential benefit of theophylline on respiratory muscle function in patients with chronic obstructive pulmonary disease (COPD) ie.

airways obstruction with or without hyperinflation. Because of the difficulty of performing precise physiological measurements in breathless individuals, and the further difficulty of inducing fatigue of the respiratory muscles, the initial study was of the action of theophylline on quadriceps muscle fatigue in normal subjects followed by studies of the action of aminophylline on bilateral diaphragmatic twitch tension in healthy volunteers and on the paced diaphragm in two patients with quadriplegia. Finally, work on the effect of the drug on respiratory muscle and quadriceps femoris strength in normal subjects and in patients with COPD was performed.

In the following section, terms of particular relevance to the following studies are defined.

DEFINITIONS

CONTRACTION: The active state of muscle, during which force is developed, and shortening may occur (Kushmerick 1983).

CONTRACTILITY: The level of muscle contraction.

FREQUENCY-FORCE CURVE (FFC): The reproducible relationship between the frequency of electrical stimulation of a muscle, and the force it develops on the ensuing contraction.

LENGTH-TENSION RELATIONSHIP: The curvilinear relationship between the starting length of a muscle and the tension achieved on its contraction. There is an optimal length or range of lengths of muscle at which greatest contraction occurs. This is normally in the relaxed state, at the length

at which stretching results in the development of passive tension, and generally corresponds to the resting length (l_o) of a muscle.

MUSCLE FATIGUE: "Loss of the capacity for developing force and/or velocity of a muscle, resulting from muscle activity under load and which is reversible by rest." (Report of the Respiratory Muscle Fatigue Workshop 1990).

MUSCLE WEAKNESS: "Impaired capacity of a resting muscle to generate force." (Report of the Respiratory Muscle Fatigue Workshop 1990).

TWITCH TENSION: The force developed by a muscle during a single contraction produced by electrical stimulation administered directly to the muscle itself or indirectly through its innervating nerve.

TYPES OF MUSCLE CONTRACTION:

VOLUNTARY: effort-dependent force development.

MAXIMAL: a contraction above which no further force development can be produced.

ISOMETRIC: a contraction during which "the muscle generates tension without shortening." (Haselgrove 1983).

ISOTONIC: a contraction during which "the muscle shortens, usually with constant velocity, while pulling against a constant load." (Haselgrove 1983).

The following historical review deals with the anatomy and physiology of the respiratory muscles, function of respiratory and other skeletal muscle during normal activity and fatigue and the effect of disease on their function. The literature with regard to the general actions of methylxanthines and particular effects on skeletal muscle and respiratory muscle is reviewed. *In vitro* work on animal muscle strips, *in vivo* animal and human studies on respiratory and other skeletal muscles are discussed in detail. The possible mechanisms of action of methylxanthines on skeletal muscle are briefly discussed.

SECTION 2 - ANATOMY AND FUNCTION OF THE RESPIRATORY MUSCLES

There are two varieties of muscle in the respiratory system - smooth or involuntary muscle which is found in the airways, and skeletal muscle which is under voluntary control and is found outside the lung in the chest wall and diaphragm. It is skeletal muscle which forms the subject of this work and with which the thesis is exclusively concerned.

CONTROL OF THE RESPIRATORY MUSCLES

The skeletal respiratory muscles are required to contract rhythmically throughout life. The respiratory centre in the brain dictates the rate of their contraction ie. the respiratory rate. Elevated pCO_2, reduced pO_2 or pH will result in activation of more motor units, and recruitment of the accessory muscles. In addition, the muscles are under voluntary control. Receptors responsive to stretch and touch within the chest-wall, diaphragm and lung also provide feedback to the central nervous system.

ANATOMY OF THE RESPIRATORY MUSCLES

ANATOMY OF THE DIAPHRAGM

The diaphragm is a dome-shaped muscular sheet, separating the trunk into thoracic and abdominal cavities. Its muscle fibres arise from the bodies and aponeurotic arches of the first, second and third lumbar vertebrae, to form the crural diaphragm, and circumferentially from the inferior six ribs to make up the costal diaphragm. These fibres are directed upwards

and are apposed to the lower rib cage. This section of the diaphragm, with its adjacent chest wall, forms the "zone of apposition" (Goldman & Mead 1973). Above this, the fibres of the diaphragm turn inwards, converging to form the central tendon (Goldman and Mead 1973, Mead 1979).

The zone of apposition increases and decreases in size with respiration and with changes in diaphragmatic configuration: at end-expiration it covers as much as a third of the surface area of the rib cage, whereas at full inspiration it covers little or nothing of the thorax (Mead 1979).

Innervation is by the phrenic nerve originating from the third to fifth cervical nerve roots. Generally the nerve roots form a single trunk in the neck, however in a proportion of the population an accessory branch from cervical nerve roots 4-6 joins the main trunk in the thorax (Gray 1989).

ANATOMY OF THE INTERCOSTAL MUSCLES

The intercostal muscles form two major groups. The superficial external group arise from the lower surface of each rib, between the tubercle and the costochondral junction, and run downward and forward to insert into the rib below. The deep internal intercostals arise from the inferior aspect of the ribs, between the costochondral junction and the posterior angle of each rib, and run downwards and backwards to the rib below. Innervation of both internal and external intercostals is by the intercostal nerves arising from first to twelfth thoracic nerve roots.

ANATOMY OF THE ACCESSORY MUSCLES

The most important accessory muscles of respiration are the scalene and the sternomastoid muscles. The scalene muscles arise from the transverse processes of the third to the seventh cervical vertebrae and insert into the first and second ribs. Innervation is from the first and second cervical nerves.

The sternomastoids arise from the anterior sternum and medial third of the clavicle and pass upwards and backwards to insert into the mastoid process and occiput. Innervation is by the eleventh cranial (accessory) and second cervical nerves.

ANATOMY OF THE ABDOMINAL MUSCLES

These comprise the external and internal oblique, the transversus abdominis, and the rectus abdominis. The external oblique arises from the inferior eight ribs and inserts into the iliac crest and together with fibres from the opposite side forms a fibrous aponeurosis as the rectus sheath in the midline, and makes up the inguinal ligament inferiorly.

The internal oblique arises from the lumbar fascia, iliac crest and the inguinal ligament, and inserts into the bottom three ribs, with fibres forming a fibrous aponeurosis in the midline. Nerve fibres from the sixth to the twelfth thoracic and the first lumbar roots provide neural innervation.

Fibres of the transversus abdominis come from the lowest six ribs, the lumbar fascia, iliac crest and inguinal ligament, and pass centrally to form another aponeurosis beneath the external and internal obliques. Innervation is by motor nerves from the seventh to the eleventh thoracic vertebrae.

The rectus abdominis passes cranially from the symphysis pubis between the internal oblique and transversus abdominis muscles to the fifth, sixth and seventh costal cartilages. Neural input comes from the lower six thoracic and the first lumbar nerve roots.

MECHANICAL ACTIONS OF THE RESPIRATORY MUSCLES

ACTIONS OF THE DIAPHRAGM

On activation of the diaphragm during inspiration, the muscle fibres shorten, pulling the dome of the diaphragm downwards against the relatively incompressible contents of the abdomen and causing abdominal pressure to rise. This increased pressure is transmitted through the zone of apposition to the lower rib cage which expands. In addition, the force of the contracting fibres on the costal insertions of the diaphragm tends to pull the lower ribs upwards. Because of the articulation and axes of the ribs, the resultant effect is to lift the circumference of the lower thorax upwards and outwards. Thus the volume of the thorax increases, producing a fall in pleural pressure relative to atmospheric pressure, and air enters the lungs.

Goldman and Mead (1973) believed that diaphragmatic contraction alone could inflate the entire thorax. This is now known not to be the case as it has been shown that solitary diaphragmatic contraction results in inward deformation of the upper rib cage, as seen in quadraplegics who have lost innervation to the intercostal muscles (Estenne & De Troyer 1985). Contraction of the intercostal muscles is required for

the upper rib cage to expand and normal inspiration to occur.

Activation of the diaphragm is posture-dependent, with greater activation when the subject is standing and sitting erect than when he is supine or leaning forward (Druz & Sharp 1982). The diaphragm inflates the lower rib cage less in the supine posture than in the upright position.

In quadriplegics seated upright, the diaphragm has an inflationary action on the lower rib cage, while on lying down the action becomes deflationary (Mortola & Sant'Ambrogio 1978, Danon et al 1979). In patients with COPD, diaphragmatic pressure development also may be posture-dependent, being relatively less when patients stand or sit erect than when they are supine or lean forward, despite increased neural input to the diaphragm in the former postures (Sharp et al 1980, Druz & Sharp 1982).

ACTIONS OF THE INTERCOSTALS

From the axis of rotation and shape of the ribs, Hamberger (1727) predicted that the external intercostals increase the cross-sectional area of the rib cage when they contract by elevating the ribs, and are therefore inspiratory in effect. In contrast, he believed that the internal intercostals, by lowering the ribs and reducing the cross-sectional area of the thorax, promote expiration.

Electromyographic studies in man support this hypothesis: normally the external intercostals contract only during inspiration, while the internal intercostals contract only during expiration (Taylor 1960, Delhez 1974).

However, depending on the lung volume both sets of intercostals can work together. In dogs at total lung capacity, the greatest tension developed tends to favour deflation of the rib cage, while at residual volume both sets of intercostal muscles tend to inflate the rib cage (Taylor 1960, Delhez 1974, De Troyer et al 1983a). The same is thought to be true in humans.

The parasternal intercostals belong anatomically to the internal intercostal group of muscles, and therefore, theoretically should be expiratory in action. This is not the case as, through the attachment of their fibres to the sternum, contraction results in equal and opposite forces which stabilise the sternum but elevate the ipsilateral ribs (De Troyer and Kelly 1982). Electromyographic activity has been recorded during respiration, and indicates activation of the parasternals in normal subjects even during quiet breathing (De Troyer and Sampson 1982).

In patients with hyperinflation and airways obstruction, persistent inspiratory intercostal contraction occurs throughout expiration and serves to maintain end-expiratory volume at a supranormal level. This has the effect of preventing airflow limitation due to closure of small airways (Martin et al 1980). Thus, the work required of the inspiratory muscles in COPD is further increased above that normally needed.

ACTIONS OF THE ACCESSORY MUSCLES

Electrical muscle activity of the scalenes has been recorded during the inspiratory phase of tidal breathing (Raper et al 1966, Delhez 1974), implying that they should be viewed as muscles of normal respiration. Together with the sternomastoids they are thought to lift and stabilize the upper rib cage (Raper et al 1966, De Troyer & Kelly 1982). Their contraction increases with ventilation (Campbell 1970).

The action of the sternomastoids is not yet defined. From studies of quadriplegics, it appears that they elevate the upper ribs (Danon et al 1979).

The accessory muscles alone can support ventilation. Quadriplegic patients with loss of diaphragmatic innervation may survive prior to artificial ventilation by sole action of the scalenes and sternomastoids. Patients with postural dyspnoea and COPD have increased activity of their scalenes, sternomastoids and parasternals during inspiration (Sharp et al 1980).

The trapezii and pectoralis minor muscles can also act as accessory muscles of inspiration. The effect on the rib cage of their contraction appears to be minor, and they probably contribute to stabilisation of the rib cage only.

ACTIONS OF THE ABDOMINAL MUSCLES

The abdominal cavity can be compared to a fluid-filled bag, and is virtually incompressible. Contraction of the abdominal muscles alone has an expiratory effect by causing abdominal pressure to rise and the diaphragm to be displaced upwards,

producing a reduction in lung volume. Their contraction also promotes expulsion during defaecation, micturition, parturition, vomiting and coughing.

During normal inspiration when the diaphragm and inspiratory intercostals contract, abdominal pressure rises opposing descent of the diaphragm, thereby acting as a fulcrum against which the diaphragm pulls upwards on the lower rib cage, and contributing to the inflationary force on the lower rib cage via the zone of apposition. During inspiration in dogs (De Troyer et al 1983b) and man (Mier et al 1985b) contraction of the abdominal muscles serves further to increase abdominal pressure, causes passive stretching of the diaphragm and thereby encourages rib cage inflation.

At the end of inspiration the still elevated abdominal pressure helps the relaxing diaphragm to return to its resting configuration, ready for the following inspiration (Grimby et al 1976, Mortola & Sant'Ambrogio 1978). In addition, contraction of the abdominal muscles tends to stretch the inspiratory muscles placing them at a more advantageous section of their length-tension relationship.

Tonic activity of the abdominal muscles changes with posture. During quiet breathing in the supine position they are electrically silent. Tone increases in the erect posture thereby helping to maintain the domed configuration of the diaphragm, and is greatest in the lower abdomen (Strohl et al 1981, Loring & Mead 1982, De Troyer 1983).

Bronchoconstriction (Martin et al 1980, Dodd et al 1984)

and diaphragmatic paralysis are both associated with increased abdominal muscle activity during expiration. This causes diaphragmatic lengthening prior to inspiration, and in addition, contributes to inspiratory pleural pressure, both actions assisting chest inflation. In the absence of diaphragmatic activity as in diaphragmatic paralysis, contraction of the abdominal muscles during inspiration may be detrimental by pulling the rib cage down and inwards, the resultant increase in abdominal pressure forcing the relaxed diaphragm upwards and producing a deflationary effect.

In contrast, in quadriplegia the effect of abdominal tone is lost and in any position but supine the diaphragm tends to descend and flatten due to gravity, lessening the effectiveness of diaphragmatic contraction (Mortola & Sant'Ambrogio 1978, Estenne & De Troyer 1985).

CONTRACTILE PROPERTIES OF THE RESPIRATORY MUSCLES

The force of contraction of the respiratory muscles is a function of four properties: the initial length of the muscle, its configuration, the velocity of shortening, and the rate at which it is stimulated.

LENGTH-TENSION RELATIONSHIP OF THE DIAPHRAGM

The length-tension relationship of skeletal muscle is a major determinant of its effect (Rohrer 1916, Rahn et al 1946). In vitro, beyond the muscle's resting length (l_o) there is an exponential relationship between skeletal muscle fibre length and the contractile tension it can develop (Ralston et al 1947, Stolov & Weilepp 1966). Beyond l_o, muscle develops

passive tension, while active tension falls. In part, this may be related to increase in twitch contraction time with muscle length beyond l_o (Bahler et al 1968). There is an optimal length, normally in the range 100-120% l_o, at which peak twitch and tetanic tension development occur (Ralston & Polissar 1949).

In vitro length-tension curves of the human respiratory muscles are similar to those for other skeletal muscles and species (McCully & Faulkner 1983). In the case of the diaphragm the optimal length for tension development covers a wider range than that of other skeletal muscle. Evanich and colleagues (1973) and Kim and co-workers (1976) studied the length-tension relationship of the feline and canine diaphragms respectively. Both groups found that as lung volume increases towards total lung capacity and the diaphragm shortens, the tension developed by the diaphragm falls. The reverse applies as lung volume decreases towards residual volume and the diaphragm lengthens. Maximal tension is developed below functional residual capacity. They postulated, therefore, that the natural resting length of the diaphragm is at a lung volume slightly below functional residual capacity. This length-tension relationship has been confirmed by in vivo studies of voluntary (Braun et al 1982) and stimulated respiratory muscle contraction (Mier et al 1985a & c) in normal subjects.

In the case of the intercostal muscles, the same relationship applies but differs slightly from that of the diaphragm. The pressure generating potential of the

parasternals is relatively greater than that of the diaphragm at high lung volumes, with the reverse true at low lung volumes, thereby ensuring that the inspiratory capacity of the inspiratory muscles remains high throughout tidal volume (Farkas et al 1985).

The length-tension relationship of muscle can adapt to its functioning range. Oliven et al (1986) have demonstrated that hamsters with elastase-induced emphysema generate maximal diaphragmatic pressure at a shorter diaphragm length than controls. This has implications for patients with hyperinflation of the lungs and flattening and shortening of the diaphragm, who by a similar adaptation may preserve diaphragmatic function. Studies in patients with chronic obstructive pulmonary disease (COPD) show that although the pressures developed by the inspiratory muscles are reduced, the reduction is not as severe as might be expected from the increase in lung volume (Byrd & Hyatt 1968, Sharp et al 1974).

DIAPHRAGMATIC CONFIGURATION

Theoretically, the dome-like shape of the diaphragm confers mechanical advantages described by LaPlace's Law:

$$P=2T/R$$

where R = radius of curvature of the diaphragm, P = pressure developed by diaphragmatic contraction, and T = tension developed by the diaphragm.

Thus as the diaphragm becomes longer and more dome-shaped, its radius of curvature becomes smaller and the pressure developed for a given tension becomes greater (Marshall 1962).

Conversely, as the diaphragm flattens with increasing lung volume, the radius of curvature becomes greater, and the pressure developed for a given tension decreases. This relationship has less influence on diaphragmatic contractility than has the length-tension characteristic, because within the normal range of tidal breathing, diaphragmatic curvature remains fairly constant (Kim et al 1976).

INTERRELATIONSHIP OF LENGTH AND CONFIGURATION

For any given lung volume the rib cage, diaphragm and abdomen may adopt a number of configurations which will depend on the dimensions of the other two parameters. Thus diaphragmatic length, and tension, at any lung volume is not fixed (Grassino et al 1978). It is important, therefore, to ensure as far as possible during studies that the chest wall is maintained in the same position, with rib cage and abdominal dimensions constant. As long as lung volume is also the same, then it can be assumed that diaphragmatic length and configuration and intercostal muscle length are similar to those of previous occasions.

VELOCITY OF MUSCLE SHORTENING

The maximum force development and velocity of shortening are both determined by myosin adenosine triphosphatase (ATPase) activity (Buchtal & Schmalbruch 1980, Saltin & Gollnick 1983). Velocity of muscle shortening is inversely related to muscle tension, so that static muscle contraction without muscle shortening results in greater tension development than does

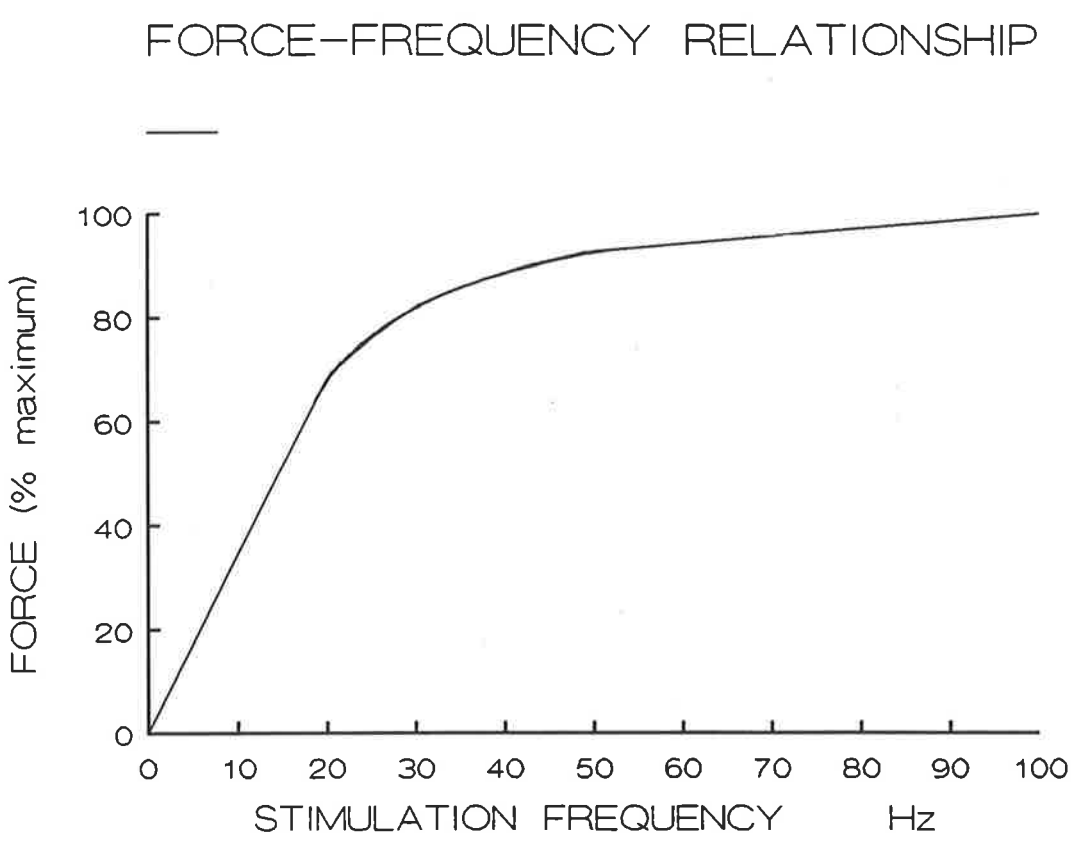
dynamic contraction with rapid and extensive muscle shortening (Fenn & Marsh 1935). For this reason, the tension produced during a maximal dynamic contraction is approximately 20% less than that during a genuinely isometric contraction (Rochester 1982). This has implications for the interpretation of studies where subjects performing isometric inspiratory manoeuvres with a bound abdomen may achieve greater pressures than those performing maximal efforts producing diaphragmatic descent and inspiratory flow. In addition, isometric muscle contraction results in the utilisation of less oxygen than an isotonic contraction because, contrary to the latter situation, during an isometric contraction no shortening occurs and no external work is performed (Hill 1938).

FREQUENCY-FORCE RELATIONSHIP

With increased frequency of muscle activation the force achieved also increases. The relationship between the frequency of stimulation of muscle, either directly or via its innervating nerve, and the tension achieved on contraction has a characteristic pattern. Edwards et al (1977a) and Moxham et al (1980, 1981) have established that similar frequency-force relationships exist for the quadriceps femoris, sternomastoid and the diaphragm.

The initial slope of the frequency-force curve is steep indicating that relatively small increments in frequency of stimulation result in large increases in tension. At high levels of stimulation the increment in tension is negligible even with large increases in frequency (Figure 1.2).

FIGURE 1.2



The frequency-force relationship of skeletal muscle. As the frequency of stimulation of the muscle increases so does the force achieved on contraction. The shape of the frequency-force curve conforms to the same pattern for a variety of skeletal muscle, including the quadriceps femoris, adductor pollicis, sternomastoid and diaphragm.

Over a frequency range of 10-30 Hertz (Hz), which is thought to correspond to the physiological firing range of neural output, the force output of the diaphragm nearly triples (Bigland and Lippold 1954, Freyschuss and Knutsson 1971, Edwards 1978, Rochester 1985). In the case of the parasternal muscles, relatively greater force is generated at high frequencies of stimulation (>50Hz) than is so with the diaphragm (Farkas et al 1985).

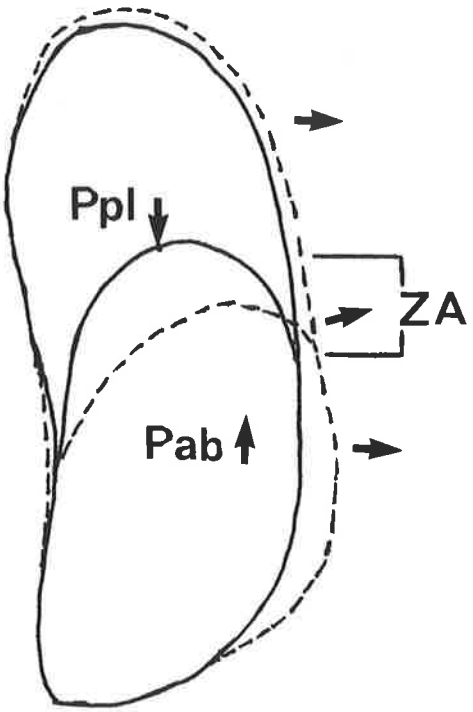
The frequency-force relationship is dependent to some extent on the length at which the muscle is operating, such that below resting length, the speed of contraction of the muscle decreases and the frequency-force relationship is shifted to the right (Farkas & Roussos 1984). Therefore, it is important that comparisons of contractions at the same frequency are also performed at the same length.

COMBINED MECHANICAL ACTION OF THE RESPIRATORY MUSCLES - NORMAL

During inspiration the diaphragm, the external intercostals and some of the accessory muscles contract. The effect of diaphragmatic contraction is to lower pleural pressure, increase intra-abdominal pressure and, through the zone of apposition, inflate the lower rib cage (Figure 1.3).

Due to the direction of insertion of diaphragmatic fibres into the lower ribs, the latter are pulled upwards as the diaphragm contracts against the incompressible abdominal contents. The net effect of the insertional and appositional forces are upward and outward (pump-handle) and forward and outward (bucket-handle) movements of the lower rib cage.

FIGURE 1.3



The effect of combined contraction of the diaphragm and other inspiratory muscles during inspiration in a healthy upright subject. The dome of the diaphragm flattens and descends, as a consequence of which pleural pressure (Ppl) decreases, abdominal pressure (Pab) increases and the abdominal wall moves outward. The lower rib cage moves outward and upward through the contraction of the diaphragm and the increase in abdominal pressure acting through the zone of apposition (ZA). Contraction of the external intercostals, the parasternals, the scalenes and the sternomastoids results in inflation of the upper rib cage.

The simultaneous contraction of external intercostal, parasternal and scalene muscles also lifts the upper rib cage upwards, forwards and outwards (Figure 1.3) (De Troyer and Kelly 1982). Particularly at high levels of ventilation, the contraction of these inspiratory muscles is coordinated to optimise diaphragmatic function (Grimby et al 1976).

Abdominal pressure and abdominal muscle contraction assist the inflationary effect of diaphragmatic contraction, return the relaxed diaphragm to its resting position during expiration and ensure restoration of favourable length and configuration to the diaphragm prior to the succeeding inspiration.

EFFECT OF POSTURE

Movement of the components of the chest wall change with posture; the rib cage expands more than the abdomen in the upright position, while the reverse is true in the supine posture. The relatively greater movement of the rib cage when upright is thought to be due to increased activity of the inspiratory intercostals and diaphragm, combined with reduced compliance of the abdomen (Druz & Sharp 1981).

COMBINED MECHANICAL ACTION OF THE RESPIRATORY MUSCLES - DISEASE

In disease, conditions occur in which the physiological and mechanical properties of the respiratory muscles may work to disadvantage.

CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

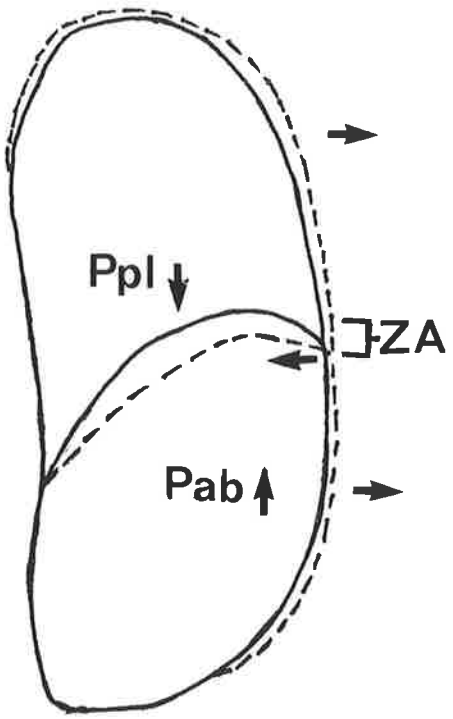
In patients with chronic bronchitis and/or emphysema and severe asthma, airflow obstruction and hyperinflation of the

lungs result in modification of the mechanical actions of the respiratory muscles, and increase both the resistive and elastic loads on the inspiratory muscles (Martin et al 1983, Fleury et al 1985).

Airflow obstruction results in prolongation of expiration, and a consequent decrease in inspiratory time. In addition, the inspiratory muscles are active during expiration as well as inspiration, thereby effectively increasing end-expiratory volume ie. functional residual capacity and preventing airflow limitation. The increase adds to the elastic work required by the inspiratory muscles during inspiration in opposing the elastic recoil force of the chest wall.

With hyperinflation, the diaphragm flattens, the insertional pull of the diaphragm on the ribs is directed more horizontally and the zone of apposition becomes smaller (Figure 1.4). In extreme hyperinflation, the appositional effects of diaphragmatic contraction and increased abdominal pressure on rib cage expansion are entirely lost, while the insertional component serves merely to pull the rib cage directly inwards, and is thought to contribute to the development of Hoover's sign. Paradoxical inward movement of the abdomen during inspiration occurs in patients with COPD, particularly those with acute respiratory failure, indicating that negative pleural pressure from intercostal muscle contraction, has been transmitted across the ineffectively contracting diaphragm (Ashutosh et al 1975, Sharp et al 1977).

FIGURE 1.4



The effect of respiratory muscle contraction in chronic obstructive pulmonary disease (COPD). The flattened muscle fibres of the diaphragm tend to pull the lower rib cage inwards rather than upwards, and because of poor tension development produce only a small decrease in pleural pressure (Ppl), and a small increase in abdominal pressure (Pab). The zone of apposition (ZA) is reduced to a minimum, and abdominal pressure is therefore transmitted only to a narrow area of the lower rib cage. The other inspiratory muscles are shortened and so capable of only poor tension development also.

The length-tension relationship of the inspiratory muscles, LaPlace's Law, and the configuration of the inspiratory muscles all dictate poor tension development in severe COPD. The result is that the maximum tension that the inspiratory muscles, and particularly the diaphragm, can develop is reduced, the effectiveness of muscle action is decreased and the work required during ventilation is increased. One consequence of this, is that in COPD the neural output to the respiratory muscles is high, comparable to that required to sustain maximal voluntary ventilation in normal subjects (Gribbin et al 1983).

EFFECT OF POSTURE

The clinical importance of these effects has been demonstrated by Druz and Sharp (1982), who studied normal subjects and patients with COPD and showed that whereas normal subjects maintained transdiaphragmatic pressure in different postures, some COPD patients had inadequate pressure development despite increased neural activation in the standing and sitting erect positions. These patients experienced dyspnoea in these positions and had some relief when they adopted the supine and leaning forward postures which were associated with the development of greater transdiaphragmatic pressures for less neural activity than in the other positions.

QUADRIPLEGIA

In quadriplegia, the intercostal and abdominal muscles are not active, the diaphragm and accessory muscles being solely functional. As a result, rib cage movement is produced by the

direct action of the diaphragm and the consequent fall in pleural pressure. The lower rib cage expands during inspiration to a lesser extent than the abdomen, and may be distorted due to greater expansion of the transverse than the anteroposterior diameter (Urmey et al 1981, Estenne & De Troyer 1985).

EFFECT OF POSTURE

When patients become supine, expansion of the transverse diameter is exaggerated and accompanied by a variable decrease in the anteroposterior diameter (Estenne & De Troyer 1985).

In both supine and seated positions, the upper rib cage may be drawn inwards during inspiration due to the effect of negative pleural pressure. There is some interindividual variation in the direction and extent of chest wall movement, which may be related to use of the accessory muscles and to spinal reflexes (Estenne & De Troyer 1985, Gutman 1973). In patients with phasic electrical activity of the scalenes during tidal breathing, paradoxical upper rib cage movement is reduced or absent, and in some cases normal outward motion may occur.

The differences seen between the seated and supine postures appear to be related to the configuration of the diaphragm. In the seated posture, large areas of the lateral and dorsal surfaces of the rib cage are covered by the apposed diaphragm, and a much smaller area of apposition exists over the anterior thoracic surface (Estenne & De Troyer 1985). This is exaggerated on assuming the supine position.

The consequent increase in the length of the diaphragm should improve the pressure-generating capacity of the

diaphragm; however, two factors may act to counteract this benefit. Firstly, abdominal compliance, which determines the effect on the lower rib cage of diaphragmatic contraction, is critical. If compliance is high, the diaphragm descends quickly, so that the relatively large velocity of shortening and the small increase in abdominal pressure result in low insertional and appositional forces on the lower rib cage. If abdominal compliance is low, as with a bound abdomen, diaphragmatic descent is opposed, and the insertional force is large as is the appositional force of increased abdominal pressure. Abdominal compliance increases on becoming supine (Konno & Mead 1968).

Secondly, gravity tends to act against the descent of the diaphragm when supine, and in the direction of descent when seated. Together these effects may make inspiratory efforts more efficient in the seated position than supine.

Quadriplegic patients may become very breathless when stood upright, because of the effect of gravity on both the abdomen and on the diaphragm itself. The diaphragm tends to shorten and flatten, thereby reducing the zone of apposition and the insertional pull of the diaphragmatic fibres on the lower ribs. Some patients are unable to stand except momentarily, although binding the abdomen to reduce compliance may assist the inspiratory action of the diaphragm.

DIAPHRAGMATIC PARALYSIS

In patients with diaphragmatic paralysis but functioning intercostal and abdominal muscles, rib cage expansion during

inspiration is relatively greater than in normal subjects for the same tidal volume. Parasternal muscle electrical activity is increased, and is associated with prolonged expiration, implying that a greater contribution to ventilation is made by the rib cage than is normal (Nochomovitz et al 1981).

In addition, abdominal muscles may be recruited during expiration with relaxation during subsequent inspiration. In the seated or standing position this manoeuvre assists passive descent of the diaphragm at the start of inspiration.

During inspiration, the negative pleural pressure resulting from intercostal muscle contraction is transmitted directly across the flaccid diaphragm, and causes paradoxical inward abdominal wall movement, an important sign of diaphragmatic paralysis.

EFFECT OF POSTURE

In the supine position, paradoxical abdominal wall movement occurs throughout the respiratory cycle, whereas in the upright posture, paradoxical motion may be confined only to late inspiration (Kreitzer et al 1978a). This difference is due to abdominal muscle contraction during expiration, and subsequent relaxation of the muscles during early inspiration, thereby allowing descent of the diaphragm in inspiration.

COMBINED DIAPHRAGMATIC PARALYSIS AND QUADRIPLEGIA

In high cord lesions, as well as the intercostal, parasternal and abdominal muscles losing innervation, so does the diaphragm. Ventilation is dependant on powerful contraction

of the scalenes and sternomastoids, but can only be sustained for short periods. Long term management is with artificial ventilation, or with phrenic nerve pacing (Glenn et al 1980).

SUMMARY

The inspiratory muscles comprise the diaphragm, external intercostals, parasternals and accessory muscles. The expiratory muscles consist of the internal intercostals.

On contraction, the diaphragm elevates abdominal pressure, and lifts and expands the lower rib cage through the zone of apposition and the muscle fibre insertions on the ribs. The upper rib cage is expanded by the action of the external intercostals, the parasternals, and during increased ventilatory effort, the sternomastoids and scalenes.

Expiration is normally a passive process. During periods of maximal ventilation or airway obstruction, expiration is assisted by contraction of the internal intercostals and the abdominal muscles, which elevate the diaphragm to its resting position.

Contraction of the abdominal muscles may also enhance inspiration by lengthening the inspiratory muscles prior to inspiration. Abdominal muscle activity is increased in the erect posture, in bronchoconstriction and in diaphragmatic paralysis.

The action of the respiratory muscles is dependant on their contractile properties. The length-tension relationship is most important. With increased length a muscle contracts more forcibly. This is relevant in hyperinflation when diaphragmatic

and inspiratory intercostal muscle fibre-lengths are short. Configuration is less important than the length-tension relationship to diaphragmatic contractility. A flat diaphragm produces less tension than does a domed diaphragm, and is a further reason for poor diaphragmatic contractility in hyperinflation.

The velocity of muscle shortening also relates to tension development. Isometric contractions and consequent minimal shortening produce greater tension than dynamic contractions with rapid muscle shortening.

Another contractile property of importance is the frequency-force relationship, which dictates increased tension development with increased frequency of stimulation.

THE RESPIRATORY MUSCLES IN DISEASE

In COPD the diaphragm is flattened, with short, horizontally-orientated muscle fibres. The external intercostal muscles are also short. During contraction, the diaphragm pulls the lower rib cage inwards, while the short muscle fibres are capable of only relatively poor tension development. The flat diaphragmatic configuration also impairs tension development, while the narrow zone of apposition allows raised abdominal pressure to influence only a small part of the lower rib cage.

The contractility of the diaphragm is often so critical that some patients find that they need to lie supine or lean forward rather than sit or stand, because greater tension can be produced in the former positions.

In quadriplegic patients with spinal cord lesions below the

second cervical vertebra, only the diaphragm and accessory muscles function. There is inward distortion of the upper rib cage. Because of the relative effects of the diaphragm, abdominal pressure and gravity, patients have most effective ventilation when seated, and least effective ventilation when erect.

In diaphragmatic paralysis, active inspiration is produced by the external intercostal and accessory muscles. The abdominal muscles also contribute by contracting during expiration, and so increasing abdominal pressure, stretching the inspiratory intercostal fibres and elevating the flaccid diaphragm, and by relaxing at the beginning of inspiration, thereby allowing passive diaphragmatic descent.

In high quadriplegia with loss of diaphragmatic innervation, ventilation can be maintained for short periods with sternomastoid and scalene contraction.

SECTION 3 - SKELETAL MUSCLE FUNCTION AND MORPHOLOGY

The functional contractile properties of the respiratory muscles conform to those of all skeletal muscle.

SKELETAL MUSCLE FUNCTION

MUSCLE ACTIVATION

Muscle can be activated either directly or through the innervating nerve. During the latter, action potentials cause release of acetylcholine from nerve end-plates into the neuromuscular junction. This in turn causes membrane depolarisation which spreads throughout the muscle, resulting in the release of calcium from myofibrillar stores. The action of calcium on adenyl cyclase catalyses the release of energy by transformation of adenosine triphosphate to adenosine diphosphate and monophosphate. The energy is utilised in the making and breaking of bridges between the protein molecules myosin and actin, which slide across each other shortening the muscle fibril.

On muscle relaxation, the actin-myosin complexes are broken allowing the molecules to slide back to their resting position.

MUSCLE CONTRACTION

TWITCH

The smallest muscle activation results in a single contraction and relaxation termed the twitch. This has a characteristic pattern common to all skeletal muscle: an initial rapid and smooth increase in tension followed by a more gradual reduction in tension to the normal resting state. The

maximum speed of decline in tension, the maximum relaxation rate, is another characteristic property of normal muscle whether activated voluntarily or by artificial stimulation (Wiles et al 1979a & b, Esau et al 1983a & b).

TETANUS

Distinct individual twitches are seen at low stimulation frequencies, but as the frequency of stimulation increases the period between the resumption of the resting state and the rise in tension of the next twitch decreases, until the muscle is unable to return completely to the resting state between contractions. This results in a prolonged, fused contraction or tetanus which, because relatively more fibres contract simultaneously, develops greater tension than a twitch.

THE FREQUENCY-FORCE RELATIONSHIP

The shape of the frequency-force relationship (see Section 2, Pages 36 & 37, Figure 1.2) is influenced by the contractile properties of muscle. Because of its slower relaxation rate, slow twitch muscle has fused contractions at a lower frequency of stimulation than fast twitch muscle.

COMPARISON OF VOLUNTARY AND STIMULATED CONTRACTIONS

Merton (1954) found that muscle can be maximally activated at will to the same extent as with a maximal tetanic stimulation. He demonstrated that a negative linear relationship exists between the level of voluntary contraction and the height of a superimposed twitch, and that no twitch can be elicited on top of a maximal voluntary contraction. The same

has been demonstrated for the diaphragm in healthy volunteers (Bellemare & Bigland-Ritchie 1984, Muscle Physiology Laboratory, unpublished observations), and in three patients with chronic airflow limitation (Newell et al 1989). Thus, although the evidence is limited in patients, measurements of transdiaphragmatic pressure during maximal inspiratory efforts can accurately reflect strength.

Physiological activation of muscle produces contractions by similar mechanisms and with similar characteristics as those produced by artificial stimulation. Equivalent amounts of energy, measured as heat production, are expended in the two types of maximal contraction, suggesting that the cellular mechanisms involved in achieving these contractions are the same (Edwards 1975). Thus, information gained in studies of artificial stimulation is relevant also to muscle activation voluntarily. However, there are differences in the types of motoneurones activated. During voluntary contraction, motoneurones to slow-twitch fibres are recruited first, at low thresholds, while on artificial stimulation motoneurones with both high and low thresholds are activated to similar degrees (Wiles et al 1979b). These differences between the two types of stimulation may result in differences in the forces achieved and the ability to maintain force.

SKELETAL MUSCLE MORPHOLOGY

Skeletal muscle consists of two components, contractile myofibrils, and non-contractile tendons and connective tissue - the latter two comprising the series-elastic component.

The myofibrils fall into two distinct morphological categories: small red and large white fibres, which are themselves subdivided into two varieties. First, the three fibre types were recognised to have different twitch rates; however, more recently they have been classified on the basis of myosin adenosine triphosphatase (ATPase) activities and the differing oxidative and glycolytic capacities of the fibres.

SKELETAL MUSCLE FIBRE TYPES

Type I - slow-twitch (red) have high oxidative capacity, low glycolytic and myosin ATPase activities and are fatigue resistant.

Type IIA - intermediate twitch have moderately high oxidative and glycolytic capacities, and myosin ATPase activity and are moderately fatigue resistant.

Type IIB - fast fibres (white) have poor oxidative but high glycolytic and myosin ATPase capacities. These fibres are fatigue sensitive.

Histochemical staining and microscopy have identified differences in mitochondrial numbers between types. The red fibres are much richer in content of myoglobin, sarcoplasma reticulum, mitochondria and fat globules than white fibres. Muscles comprise varying percentages of the three muscle fibre types and the relative proportions dictate the contractile properties. Type I fibres are recruited early at low levels of contraction, while Type II fibres are progressively recruited at higher levels (Garnett et al 1978). The differing percentages of each fibre type thus reflect the relative

requirements for prolonged and/or forceful contraction.

ENERGY SUPPLY

Energy stores in muscle fibres consist of adenosine triphosphate (ATP) and phosphorylcreatinine which are utilised during muscle contractions lasting less than 10 seconds (Edwards 1978). Activities extending beyond this for up to 45 seconds require metabolism of glycogen, and for longer bursts of activity, chemicals and oxygen for energy production are provided from the blood (Edwards 1978). Blood flow is impaired during muscle contraction and energy repletion and removal of waste occurs primarily during relaxation.

RESPIRATORY MUSCLE MORPHOLOGY - NORMAL

The diaphragm and intercostal muscles have a predominance of Type I fibres (55%) (Lieberman et al 1973). The endurance properties of the diaphragm reflect this (Gandevia et al 1983), making it relatively fatigue resistant in keeping with its function of continuous rhythmical contraction. The twitch characteristics are intermediate in type (Pagala et al 1984).

The oxidative capacity of the diaphragm and its capacity for increased blood flow during exercise or loading exceeds those for most limb muscles (Rochester & Bettini 1976, Robertson et al 1977, Faulkner et al 1979).

RESPIRATORY MUSCLE MORPHOLOGY - DISEASE

Diaphragmatic muscle fibre size is increased in emphysema (Scott & Hoy 1976), although total muscle bulk appears to be decreased (Fromme 1916, Steele & Heard 1973, Thurlbeck 1978).

Butler (1976) found a significant inverse relationship between diaphragmatic area and the extent of emphysema. Openbrier et al (1983) found that reduction in body weight is related to the reduction in airway calibre and transfer factor, and associated with the corresponding reduction in diaphragmatic weight. In contrast, patients of normal weight and diagnosed as having COPD, have reduced diaphragmatic muscle fibre size (Hughes et al 1983).

Respiratory muscle appears to adapt to loads and short operating lengths by decreasing the number of sarcomeres (Supinski & Kelsen 1982, Farkas & Roussos 1983), however in COPD, this is likely to compensate only partially for the poor maximum tension development related to the loss of muscle bulk and the short muscle length consequent on increased end-expiratory volume (Rochester & Braun 1985).

Sanchez and colleagues (1982,1985) have shown that the number of Type I & II fibres decrease in proportion to airway flow, reducing the endurance potential of the diaphragm in emphysematous patients. In addition, the metabolism of respiratory muscle changes with the degree of hyperinflation and airways obstruction (Sanchez et al 1984), and this also may reduce the resistance to fatigue (Holloszy & Booth 1976).

Consequently, the diaphragm in patients with airways obstruction and hyperinflation is incapable of developing normal strength, and also may be more susceptible to the development of fatigue.

In high quadriplegia, resulting in loss of diaphragmatic

innervation, muscle wasting arising from lack of use affects the diaphragm as well as other skeletal muscle (Glenn et al 1984). It is thought that following the initiation of phrenic nerve pacing, a training regimen improves the endurance of the diaphragm and reverses diaphragmatic muscle atrophy (Nochomovitz et al 1984, Garrido et al 1987).

SKELETAL MUSCLE DYSFUNCTION

Disruption of skeletal muscle function or morphology due to failure of neuromuscular transmission or of propogation of action potentials within muscle, impaired blood supply, or pathology of the muscle itself, will result in weakness or fatigue. Any of these may also result in dysfunction of the respiratory muscles.

RESPIRATORY MUSCLE WEAKNESS

Weakness is defined as impaired capacity of a resting muscle to generate force (Report of the Respiratory Muscle Fatigue Workshop 1990).

CENTRAL WEAKNESS

Any central nervous system lesion which affects the respiratory centre may result in impairment of the automatic ventilatory neural drive, causing central alveolar hypoventilation. In the classic example of this, Ondine's curse (which is usually fatal in infancy) respiratory centre neurones fail to fire during sleep (Severinghaus & Mitchell 1962). In recent years, frequent reports of sleep apnoea have been published. In this condition, the reduction in upper airways

tone, seen during rapid eye movement sleep, and diminution of ventilation combine in susceptible individuals to produce cyanosis and hypoxia.

The central demyelinating disorder, multiple sclerosis, is well known to produce ventilatory failure as a terminal event (Guthrie et al 1952, Cooper et al 1985a), but also has been identified in patients relatively early in their disease (Mier et al 1988a). Patients with disorders producing cerebellar atrophy, namely Friedrich's ataxia and hereditary spastic paraplegia, have been found to suffer diaphragmatic weakness (Mier-Jedrzejowicz & Green 1988). Impaired ventilatory response to breathing CO_2 (Kilburn et al 1959) and sensitivity to anaesthetic agents (Gillam et al 1964) have been found in myotonic dystrophy, suggesting central nervous system pathology as well as the muscular abnormality.

SPINAL CORD LESIONS

Diseases of the motor neurones can result in respiratory muscle failure if the anterior horn cells innervating the phrenic or other respiratory muscle nerves are affected. The most common cause was poliomyelitis, until adequate vaccination largely eradicated it. In addition to producing gradual onset of respiratory failure because of the effects of the kyphoscoliosis on respiratory muscle function, lung volumes and pulmonary vasculature, poliomyelitis also results in death from ventilatory failure due to destruction of spinal and bulbar motor neurones during the infective illness itself (Sarnoff et al 1951).

Respiratory muscle weakness has been documented to occur early in amyotrophic lateral sclerosis, passing unrecognised on routine lung function measurements (Kreitzer et al 1978b, Serisier et al 1982). Spinal muscular atrophy is a complex of rare hereditary disorders which, depending on the type of anterior horn cell involved, may cause diaphragmatic weakness (Newsom Davis et al 1976).

PERIPHERAL NEURAL DEFECTS

Peripheral neuropathies may involve any or all of the respiratory nerves. The cause of isolated phrenic neuropathy is often obscure. Unilateral phrenic nerve palsy may produce respiratory muscle weakness (Fackler et al 1967), although bilateral phrenic nerve palsy is more likely to result in ventilatory deficit.

In patients with Guillain-Barre syndrome, although demyelination of peripheral nerves is transitory, ventilatory failure is common, occurring in up to 50% of cases (Ravn 1967, Gourie-Devi & Ganapathy 1985). Artificial ventilation may be required to restore blood gases to normal, and until the respiratory muscles can maintain ventilation (Braun et al 1983).

Other causes of respiratory neuropathy include injury (Larson & Evans 1963), cooling or damage at cardiothoracic operation (Marco et al 1977, Wheeler et al 1985, Driver et al 1987, Burgess et al 1989), neuralgic amyotrophy (Cape & Fincham 1965), immunolgical response to anti-tetanus immunoglobulin (Smith & Smith 1955, McCredie et al 1962), and herpes zoster

infection (Brostoff 1966, Dutt 1970, Passerini et al 1985).

Weakness has been reported in the hereditary motor and sensory neuropathy (Charcot-Marie-Tooth disease) (Laroche et al 1988a). In two families, diabetes mellitus also may have played a contributory role (Blythe et al 1977, Chan et al 1987). An isolated lesion of genetic origin also has been identified to produce diaphragmatic weakness in homozygote twin brothers (Molho et al 1987).

MUSCLE PATHOLOGY

Cachexia results in loss of respiratory muscle (Arora & Rochester 1982), as does emphysema (Thurlbeck 1978) and COPD despite maintenance of normal body weight (Sanchez et al 1982, Hughes et al 1983). In addition, hypercapnia has been recognised to cause impaired diaphragmatic contractility (Juan et al 1984).

Metabolic disorders such as hyperthyroidism produce respiratory muscle weakness. Patients with thyrotoxicosis with abnormal lung function and respiratory muscle tests showed improvement in these parameters on adequate treatment (Stein et al 1961, Mier et al 1989). Reduced respiratory muscle strength occurs with myxoedema and similarly improves with treatment (Weiner et al 1986). The glycogen storage disease, acid maltase deficiency, causes weakness (Newsom Davis 1976) through muscle replacement.

Atrophy may arise due to disuse (Booth & Kelso 1973, Witzmann et al 1983), and is well documented to occur in the diaphragm and intercostal muscles after paralysis following

cervical spinal injury (Glenn et al 1984) and other neuropathic disorders.

Muscular dystrophies result in destruction of muscle fibres, and work on dystrophic hamsters has found that the collagen content of the diaphragm is increased at the expense of normal muscle (Al-Zaid et al 1990). Respiratory muscle weakness has been reported early in the course of Duchenne muscular dystrophy (Inkley et al 1974, Smith et al 1987), limb-girdle dystrophy (Newsom Davis et al 1976) and in myotonic dystrophy (Serisier et al 1982).

In connective tissue disorders, muscle may be disrupted by fibrosis or inflammation. In scleroderma, cryptogenic fibrosing alveolitis and systemic lupus erythematosus, respiratory strength may be preserved (Brophy et al 1986, Laroche & Green 1988), or reduced (Gibson et al 1977, De Troyer & Yernault 1980, Chausow et al 1984). In sarcoidosis, granuloma have been identified in the diaphragm (Pandya et al 1988), and the muscle disruption is thought to contribute to dyspnoea. Respiratory muscle involvement has been identified in mixed connective tissue disease (Martens & Demedts 1982) and in Still's disease (Braidy & Poulson 1984).

Viral infections have been found to cause transient reduction in respiratory muscle strength which recovered by the 14th day of the illness (Mier-Jedrzejowicz et al 1988b); such reduction may be part of a generalised myopathy.

DISRUPTION OF ELECTRICAL ACTIVATION OR CONTRACTION

Impairment of neuromuscular transmission causes the weakness

seen in myaesthenia gravis, muscle cooling and partial curarization (Heisterkamp et al 1969). The most common disorder of neuromuscular transmission to affect the respiratory muscles is myaestheia gravis (Mier-Jedrzejowicz et al 1988c). Blockage of neuromuscular transmission can be induced by injection of curare and produces the clinical picture of myaesthenia gravis, while patients with rheumatoid arthritis or Wilson's disease treated with penicillamine have been reported to develop a similar condition (Masters et al 1977). Lambert-Eaton myaesthenic syndrome in association with bronchial carcinoma may affect the respiratory muscles (Wilcox et al 1988).

In ischaemia and myotonia congenita (Wiles & Edwards 1977) postganglionic membrane function is disrupted, while abnormalities of energy provision or exchange may also affect the contractile process. Some of the dysfunction in COPD may be due to a decrease in the maximal enzymatic metabolic activities of hexokinase and lactic dehydrogenase (Sanchez et al 1984), while glycogen depletion has been identified in such patients during acute respiratory failure (Gertz et al 1977).

Excitation-contraction coupling is thought to be the pathological mechanism producing weakness in both familial hypokalaemic periodic paralysis (Engel & Lambert 1969) and myotonia (Wiles & Edwards 1977). In myotonia congenita, respiratory muscle bulk may be normal or increased, however normal muscle relaxation is prolonged and may be associated with weakness (Estenne et al 1984).

Reversal of generalised abnormalities in biochemistry have been shown to result in improvements in respiratory muscle

function. Reports cite deficiencies in magnesium (Dhingra et al 1984) phosphate and calcium (Aubier et al 1985a & b) as causing measurable degrees of respiratory muscle weakness. Phosphate depletion is thought to contribute to muscle weakness and fatigue in COPD patients with respiratory failure (Aubier et al 1985a).

MECHANICAL DISADVANTAGE

The sensitivity of respiratory muscle length-tension relationships to lung volume has been demonstrated in normal subjects (Braun et al 1982, Mier et al 1985a & c) and in patients with COPD (Dodd et al 1984). In COPD, hyperinflation causes the inspiratory muscles to contract from a position on the length-tension curve which dictates poor tension development (Rohrer 1916, Rahn et al 1946).

Low chest wall and lung compliance from fibrosis of the rib cage or lung as in cryptogenic fibrosing alveolitis, systemic lupus erythematosis and other connective tissue disorders, necessitates strong inspiratory muscle contraction to inflate the stiff rib cage. Many patients retain normal inspiratory muscle function (Brophy et al 1986, Laroche & Green 1988), although others show weakness (Gibson et al 1977).

SKELETAL MUSCLE FATIGUE

Fatigue is defined as "the condition in which there is loss of the capacity for developing force and/or velocity of a muscle, resulting from muscle activity under load and which is reversible by rest" (Report of the Respiratory Muscle Fatigue

Workshop 1990).

CENTRAL FATIGUE

During a sustained strong voluntary contraction there is a prompt decline in neural discharge from high levels (100Hz) at the beginning of muscle activation, to much lower levels of discharge during prolonged contraction (Edwards 1978). Even then, neural firing frequencies only exceed 30Hz for a few seconds (Jones et al 1979). Bigland-Ritchie and colleagues (1978) showed that when an apparently maximal voluntary contraction (MVC) declined, renewed effort resulted in an increase to the previous maximum level. This reversible decline in tension is termed central fatigue.

Central fatigue contributes to the fall in force sometimes seen during assessment of respiratory muscle strength and maximal voluntary ventilation, when the volitional component of the test is large, and when maintenance of high firing frequencies is required.

PERIPHERAL FATIGUE

Definition of the frequency-force relationship has allowed the differentiation of two types of peripheral fatigue: high and low frequency fatigue. In the former, force at high frequencies cannot be sustained, while force developed at low frequencies of stimulation is normal. It arises when neuromuscular transmission is impaired. Anoxia and impaired energy exchange may affect the process of membrane depolarisation, causing high frequency fatigue (Spande & Schottelius 1970, Edwards 1978).

In low frequency fatigue, there is selective loss of force at low frequencies with maintenance of normal force generation at high frequencies. Muscle contraction does not occur despite adequate neural output, transmission across the neuromuscular junction and activation of the motor end-plate. This is termed failure of excitation-contraction coupling and is thought to arise secondary to failure of calcium release or transmission within the muscle cell (Kugelberg and Lindegren 1979).

Peripheral fatigue may be manifest as either failure of generalised muscle performance or failure only of the more fatigue-sensitive (Type II) muscle fibres. Despite the high proportion of fatigue-resistant Type I fibres in the diaphragm, it is thought that fatigue can occur under certain conditions.

RESPIRATORY MUSCLE FATIGUE

Fatigue may arise when the blood supply to the respiratory muscles is impaired, or when the work of breathing becomes excessive for the capacity of the muscle. The latter occurs when the diaphragm is required to develop a force above a certain percentage of maximum to maintain ventilation (Roussos & Macklem 1977). Experimentally, this situation has been produced in normals and in patients with COPD required to breath through high inspiratory resistances (Roussos et al 1979, Bellemare & Grassino 1983). It also occurs when the duration of inspiratory muscle contraction exceeds a certain percentage of breath cycle time (Kelsen & Nochomovitz 1982, Bellemare & Grassino 1982a), so shortening the time available for restoration of energy stores.

These two factors together determine the time taken for the diaphragm to fatigue (Tlim) and define the tension-time index of the diaphragm (TTdi). Beyond a critical value, fatigue will certainly occur (Bellemare & Grassino 1982a).

RESPIRATORY MUSCLE FATIGUE IN COPD

Hyperinflation maintained by inspiratory muscle activity, as is the case in COPD, has a number of effects on the inspiratory muscles. Inspiratory muscle contraction time is increased in order to increase the position of functional residual capacity. The elastic recoil of the chest wall is increased, necessitating greater tension development by the inspiratory muscles to overcome it. The tension-time index of the inspiratory muscles in patients with COPD is, therefore, greater than in normal subjects.

In addition, the flattened diaphragm is capable of relatively poor tension development for its length, and the reduction in respiratory muscle strength increases the risk that the critical TTdi will be exceeded, and Tlim will be reached. Furthermore, hypoxia by reducing energy supplies, appears to predispose to fatigue (Roussos & Macklem 1977, Jardim et al 1981, Aubier et al 1981b), while hypercapnia also increases the risk by directly impairing diaphragmatic contractility (Juan et al 1984).

As fatiguing work continues, central nervous system firing frequency increases to maintain contractile force, and ultimately falls as does contractility (Merton 1954). The high levels of neural output noted in patients with COPD may relate

to the performance of near-fatiguing work by the inspiratory muscles (Gribbin et al 1983).

Various groups have produced respiratory muscle fatigue *in vivo* utilising these principles; in dogs through the production of cardiogenic shock (Aubier et al 1981b), and in sheep and man after breathing through inspiratory resistances and causing hyperinflation (Bazzy & Haddad 1984, Roussos et al 1979). Low frequency fatigue has been produced in patients with airway obstruction who are required to breath through an added resistance (Moxham et al 1980, 1981).

These studies found that as inspiratory time became prolonged, respiration became more rapid, further increasing the TT_{di} and, in addition, shortening blood delivery time and threatening energy supply.

For these reasons, patients with hyperinflation and increased airways resistance from emphysema and airways obstruction are thought to be at high risk of developing respiratory muscle fatigue (Bellemare & Grassino 1983).

PREVENTION OF FATIGUE IN COPD

It has been postulated that by reducing the percentage of maximum diaphragmatic contraction needed for ventilation, and therefore the work of breathing, fatigue can be avoided (Rochester 1981). Gottfried et al (1988) have shown that both ventilation of severe COPD patients with either continuous positive airway pressure or constant negative extrathoracic pressure reduces the tidal excursions of the diaphragm with little change in end-expiratory volume, and in addition,

reduces the oxygen cost of breathing.

Prior to the performance of this thesis, the demonstration of an increase in diaphragmatic twitch and protection from low frequency fatigue of the diaphragm in normal subjects with aminophylline, raised the hope that such muscle effects might be also seen in patients with COPD (Aubier et al 1981a).

Alternative or additional beneficial actions such as reduction in the degree of hyperinflation and/or airways resistance might increase the relative efficiency of the respiratory muscles (Loring & Mead 1982), and thereby decrease the work of breathing. Either type of effect could be of relevance in the prevention of respiratory failure in COPD.

RESPIRATORY MUSCLE FATIGUE IN QUADRIPLEGIA

A further group believed to be at risk of diaphragmatic fatigue are quadriplegic patients receiving phrenic nerve pacing (Glenn et al 1980). Patients with complete cervical cord lesions above the third cervical vertebra have no neural output to the diaphragm, intercostal or abdominal muscles, and require continuous artificial ventilation to live. Loss of innervation and immobilisation result in atrophy of skeletal muscle (Booth & Kelso 1973, Witzmann et al 1983), seen in the limb muscles of quadraplegics, and thought to occur with equal severity in the respiratory muscles (Glenn et al 1984).

Patients with high cord lesions and intact phrenic nerves, can achieve independence from artificial mechanical ventilation after implantation of radiotransmitters to activate the viable phrenic nerves electrically. In some patients continuous 24

hour pacing is possible in two 12-hour shifts pacing each hemidiaphragm (Glenn et al 1980, 1986, Garrido et al 1987). The identification of optimal pacing characteristics and conditioning of the diaphragm have increased the chances of continuous paced ventilation being successful (Oda et al 1981, Glenn et al 1984, Nochomovitz et al 1984, Garrido et al 1987). Ineffective diaphragm pacing has been reported, and is thought to be caused either by failure of neuromuscular transmission or by muscle fatigue which may result in irreversible damage to the muscle (Glenn et al 1980, Oda et al 1981).

PREVENTION OF FATIGUE IN QUADRIPLEGIA

A regimen of electrical stimulation of the phrenic nerves, closely mimicking physiological neural activation, has been advocated to reduce the risk of neuromuscular failure (Oda et al 1981, Glenn et al 1984). In addition, a programme of diaphragmatic conditioning has been found to improve strength of contraction over a period of weeks, and is believed to be analogous to limb muscle training in athletes (Nochomowitz et al 1984).

Nevertheless, in some patients, upper rib cage indrawing produced by solitary diaphragmatic contraction may counterbalance the inflationary effects, resulting in inadequate tidal volume. The high pressures needed to offset this chest wall distortion cannot always be sustained, and a period of conventional ventilation may be required each day to allow the diaphragm to rest (Glenn et al 1980). It has been suggested that augmentation of diaphragmatic contraction with

methylxanthines may enable these patients to achieve independence from mechanical ventilation (Nochomowitz et al 1984).

SUMMARY

At the lowest level of stimulation, muscle contraction results in a single twitch. Increased frequency of stimulation produces twitches at shorter intervals, until contractions fuse to form a tetanus.

Muscle can be activated either by artificial stimulation or voluntarily. The magnitude of the maximum contraction by either method is the same. The metabolism of muscle during the two types of contraction is also the same. Thus, studies of artificial stimulation have relevance to normal voluntary muscle activity.

Skeletal muscle function is dependant on the morphology. Inspiratory muscles comprise predominantly slow twitch Type I (fatigue-resistant) muscle fibres. In COPD, the risk of weakness and fatigue is due partly to reduction in respiratory muscle bulk and the numbers of fatigue-resistant fibres. Similarly, morphology may alter with function. Some compensation for the hyperinflation of COPD is provided by resorption of sarcomeres, and the consequent lengthening of the remaining myofibrils.

Weakness and fatigue arise because of impairment of neural input, disruption of the muscle itself, or mechanical disadvantage. Low frequency fatigue, in which adequate electrical activation fails to result in muscle contraction,

results from poor or insufficient energy supply for the required work output.

The muscle fibre type, a variety of skeletal muscle functional properties (length, configuration, frequency of activation, whether contracting statically or dynamically) and external factors (hypoxia, hypercapnia, hyperinflation, compromised blood supply) all influence the level of muscle contraction, and the likelihood of fatigue-development.

The major determinants of low frequency fatigue are the force of muscle contraction as a percentage of strength, and the duration of contraction (Bellemare & Grassino 1982a). Muscle weakness renders the muscle more susceptible to fatigue because it decreases the strength of the muscle relative to the force routinely required from the muscle.

In COPD, respiratory muscle strength is reduced due to hyperinflation, hypoxia, hypercapnia, muscle wasting and a relative reduction in the number of Type I fibres. Inspiratory muscle work is increased because of elevated airway resistance and raised elastic forces.

In quadriplegia, muscle atrophy impairs strength, and paradoxical movement of the rib cage and abdomen decreases the efficiency of the diaphragm, resulting in the requirement for a greater than normal level of diaphragmatic contraction to maintain ventilation.

SECTION 4 - ACTIONS OF METHYLXANTHINES

There are a variety of methods of treatment of respiratory muscle weakness and fatigue. This thesis focusses on the possible actions of methylxanthines at accepted therapeutic doses on respiratory muscle contraction. Other actions of methylxanthines which may result indirectly in an effect on the respiratory muscles are discussed briefly.

METHYLXANTHINES

Methylxanthines are naturally occurring alkaloids originally derived from plants, and used throughout the world as stimulants. Caffeine (1,3,7-trimethylxanthine) and theophylline (3,7-dimethylxanthine) are the two most widely occurring xanthine derivatives. Theophylline is used most extensively pharmaceutically, while caffeine is found in coffee, tea, cocoa, chocolate, soft drinks and some proprietary therapeutic compounds.

NON-SKELETAL MUSCLE ACTIONS

RESPIRATORY EFFECTS

There is dispute about the action of aminophylline on normal airways, with improvement in specific airways conductance found in one study (Mackay et al 1983), and no change in airways resistance reported in another (Estenne et al 1980). Clinically, there is improvement in symptoms, vital capacity (VC) and airway calibre (Rossing et al 1980, Bukowsky et al 1984), although these benefits appear to be more apparent in asthmatics than in patients with chronic bronchitis (Alexander et al 1980, Eaton et al 1982). These actions form

the rationale for the use of theophylline in acute asthma and COPD.

Stimulation of respiration has been described frequently, and has been attributed to an effect on the central nervous system. In 1913 Cushny noted that, with theophylline, although vagotomy in rats produced a rise in respiratory rate, tidal volume was unchanged. LeMessurier (1936) found that the effect on minute volume of caffeine was not altered by ablating both carotid sinuses and vagi, suggesting a central mechanism, and Richmond (1949) reported that caffeine, and to a lesser extent aminophylline, increased ventilation during inhalation of air and CO_2 enriched mixtures. He also proposed a central mechanism.

In normal subjects, ventilatory response to CO_2 rebreathing is enhanced, and respiratory minute volume is increased at all levels of pCO_2 (Stroud et al 1955). Theophylline appears to be more potent than caffeine in this respect. Galdston & Geller (1957) maintained that the threshold of CO_2 ventilatory response was lowered, but that the slope of the response remained the same.

As well as altering the response to CO_2, methylxanthines cause hypoxic ventilatory drive to increase (Lakshminarayan et al 1978, Sanders et al 1980). Increased ventilation has been shown in patients with Cheyne-Stokes respiration (Dowell et al 1965), with heart-failure, and in premature babies and older infants at risk of apnoea (Kuzemko & Paala 1973, Davi et al 1978, Gerhardt et al 1979), although the latter has been

disputed (Milerad 1987).

Other respiratory actions include inhibition of mast cell degranulation *in vitro* (Baxter et al 1972, Marquardt et al 1978), and enhancement of mucociliary clearance (Sutton et al 1981, Cotromanes et al 1985).

CENTRAL NERVOUS SYSTEM EFFECTS

Central stimulation with caffeine and aminophylline has been recognised for many years (Sollman & Pilcher 1911, LeMessurier 1936, Richmond 1949, Lundberg et al 1981). It is thought to influence the respiratory system by increasing both respiratory rate and tidal volume.

There are believed to be more general actions on the central nervous system also. Caffeine's effects have been studied at doses consumed in beverages, and increased capacity for sustained intellectual effort and decreased reaction time have been noted (Curatolo & Robertson 1983). However, actions requiring delicate muscular coordination can be impaired (Goldstein et al 1965).

Methylxanthines reverse the central depression of benzodiazepines and methadone (Olsen & Schlitt 1981, Wangler & Kilpatrick 1985). Asthmatics on therapeutic doses have increased serial digit learning, motor unsteadiness, sleep disturbance and depression (Joad et al 1986). At high doses nervousness, tremor, restlessness and irritability occur, and later, convulsions. Convulsions can occur at theophylline levels as low as 30mcg/l (Kordash et al 1977). Nausea and vomiting are central effects and occur with intravenous as well

as oral administration. All central effects are dose related.

CARDIOVASCULAR SYSTEM EFFECTS

Effects on the cardiovascular system also vary with dose. Sollmann & Pilcher (1911) were amongst the first to perform definitive studies on the action of caffeine in dogs and cats in vivo. They identified a fall in blood pressure, attributed to a direct effect on the heart, although under some circumstances, depending on the dose and speed of injection, they found a rise. At high doses cardiac arrythmias and death occurred.

Krop (1944) investigated the actions of caffeine, aminophylline and theobromine on cat papillary muscle, and found that all three agents augmented cardiac muscle contraction although the action was rapid and short-lived. Marcus et al (1972) confirmed that cardiac muscle contractility was enhanced by methylxanthines, and suggested that this was due partly to a direct action and partly to an indirect action by provoking catecholamine release.

Starr et al (1937) studied patients with cardiac disorders and noted increased cardiac output and work, and decreased peripheral vascular resistance with aminophylline. They found little change in pulse rate, blood pressure or respiratory rate. For years, theophylline was advocated in heart failure because of the improvement in cardiac output and decrease in left ventricular filling pressure (Starr 1955, Ogilvie et al 1977). Right and left ventricular ejection fractions are increased in patients with COPD, even at low therapeutic

concentrations of theophylline (Matthay et al 1978, 1982). Heart rate and ventricular irritability are also increased, but with no increase in ventricular premature beats (Patel et al 1981). Sudden death after intravenous administration of theophylline is a well known risk in such patients.

The implications of these actions on the respiratory system are that blood flow to the respiratory muscles may be enhanced by methylxanthines, resulting in an increase in supply of nutrients and oxygen and in removal of the products of muscle metabolism, both of which may assist respiratory muscle contractility or help to reduce the possibility of fatigue.

SMOOTH MUSCLE EFFECTS

Effects on vascular smooth muscle are variable. Generally, methylxanthines relax the vascular smooth muscle contraction produced by catecholamines (Hedqvist et al 1978). However in some circumstances contraction may be enhanced. Cerebral blood flow is diminished (Weschler et al 1950, Moyer et al 1952).

The most important effect is bronchial smooth muscle relaxation, which is the basis for the use in respiratory disease. The smooth muscle relaxation is enhanced by prior contraction with acetylcholine or histamine, and may be increased by simultaneous administration of sympathomimetics (Foster 1966, James 1967, Bertelli et al 1973, Wolfe et al 1978, Ziment & Steen 1978).

ENDOCRINE EFFECTS

Methylxanthines increase concentrations of catecholamines in plasma and in organs previously depleted of stores by promoting

release from the adrenal medulla (Berkowitz & Spector 1971, Poisner 1973, Snider & Waldeck 1974). Unlike other effects, this action does not require the presence of extracellular calcium (Peach 1972). Plasma renin activity is enhanced after infusion of methylxanthines (Robertson et al 1978).

Thus, effects of methylxanthines on the heart and respiratory muscles may not arise directly, but may be mediated through catecholamine release.

METABOLIC EFFECTS

Caffeine is reported to increase metabolic rate. Theophylline increases plasma free fatty acids and glycerol, and inhibits muscle glycogenolysis, in dogs (Bellet et al 1968) and man (Arnman et al 1975).

This action may improve the supply of energy to respiratory muscle, and so enhance muscle contractility and/or protect against the development of fatigue.

THERAPEUTIC CONCENTRATIONS

Safe therapeutic concentrations of theophylline are in the range 10-20 mg/l; an increase in pulmonary function is seen in asthmatics at 3-25mg/l (Mitenko & Ogilvie 1973), although at concentrations below 10mg/l the effects are small. Above this range serious toxic effects are observed (Jacobs et al 1976). Minor adverse effects such as headache, nausea and abdominal discomfort occur within these limits, and 4% of adults cannot tolerate even low serum levels (Greening et al 1981).

ABSORPTION AND METABOLISM

Methylxanthines can be administered via the oral, rectal or parenteral routes. With oral slow-release preparations, blood levels in the therapeutic range can be achieved for nearly 24 hours after a single dose. Peak concentrations occur 4-6 hours after dosing, and the trough level 10-12 hours post-dose with formulations given twice daily. Ingestion at night (Scott et al 1981) and with food slows absorption into the circulation and side effects are reduced.

Rectal administration conveys no advantage as absorption is poor and less predictable than with oral administration, and side effects, even gastro-intestinal irritation, still occur. Intravenous infusion can be given but should be slow, and intramuscular injection is too painful to be advocated.

Metabolism of theophylline is primarily by dealkylation and hydroxylation in the liver, but a small proportion is excreted unchanged in the urine. The half life of theophylline in adults is eight hours. Elimination is faster in children (Jusko et al 1977), in smokers (Powell et al 1977), in men and in women on the oral contraceptive pill, and is slowed in pneumonia (Powell et al 1978), right heart and hepatic failure (Piafsky et al 1977). Drugs such as cimetidine (Reitberg et al 1981) and erythromicin (Kozak et al 1977) can interfere with metabolism.

Wienberger and Ginchansky (1977) have shown that accurate dosage prediction can be difficult, and small increments in dose may result in higher than expected blood levels. For this reason and because of the narrow therapeutic window, dose titration is advocated when introducing methylxanthines or

changing dose.

SUMMARY

Methylxanthines have effects on multiple organ systems. They produce bronchodilatation by respiratory smooth muscle relaxation. This occurs to a greater extent in patients with asthma than with COPD and fixed airway disease; a response difference reflected in the conflicting reports of clinical benefit in COPD.

Tidal volume and respiratory rate increase and ventilatory responses to hypercapnia and hypoxia are enhanced, via central nervous system stimulation. The latter is also responsible for many of the side effects of methylxanthine treatment.

Stimulation of the cardiovascular system occurs with augmentation of cardiac muscle contractility, thought to be due, in part, to a direct effect and, in part, to catecholamines released from the adrenal medulla. Cardiac output and work are increased and peripheral vascular resistance are decreased. All these actions may result in enhancement of blood supply to the respiratory muscles.

Methylxanthines also increase concentrations of metabolic substrates in the blood stream, thereby increasing energy supplies to the respiratory muscle.

SKELETAL MUSCLE ACTIONS

sIN VITRO STUDIES

EFFECT ON MUSCLE STRIPS

Methylxanthines have for many years been known to enhance

skeletal muscle tension. Zunz (1932) reported that direct stimulation of frog muscle resulted in increased contraction after exposure to methylxanthines. Caffeine prolongs the active state of contraction (Ritchie 1954, Axelsson & Thesleff 1968). Axelsson & Thesleff (1968) were able to detect different effects at different caffeine levels. They found that frog skeletal muscle twitch tension was potentiated at low concentrations, and contracture was produced at high concentrations. This has been confirmed for both caffeine and theophylline in a variety of studies on skeletal muscle strips (Luttgau & Oetliker 1968, Sandow & Seaman 1964, Sandow & Preiser 1964, Sandow et al 1965, Sandow & Brust 1966, Yamaguchi 1975), and on diaphragm muscle strips from animals and humans (Kramer & Wells 1980, Howell et al 1981, Wittman & Kelsen 1982, Jones et al 1982).

INFLUENCE OF MUSCLE MORPHOLOGY

Responses depend on fibre type: there is a greater effect on slow-twitch fibres than on fast-twitch or intermediate-twitch fibres (Brust 1976).

INFLUENCE OF MUSCLE LENGTH

Methylxanthines produce greatest effects in short fibres (Lopez et al 1981)

INFLUENCE OF THE FREQUENCY OF STIMULATION

Twitches produced with both direct and indirect stimulation are enhanced (Varagic & Zugic 1971), however the site and frequency of stimulation can influence the response seen.

Wittman and Kelsen (1982) studied tension developed in hamster diaphragm muscle strips exposed to caffeine concentrations of one millimolar (mM) during stimulation at a range of frequencies. Caffeine potentiated tension produced below 50Hz on direct and indirect stimulation. At higher frequencies, caffeine potentiated tension on direct stimulation, but on indirect stimulation there was a fall in tension. This was attributed to impairment of neuromuscular transmission at high frequencies of stimulation. The effects can be modified by adrenaline which further augments skeletal muscle tension (Varagic & Zugic 1971).

Supinski et al (1986) noted that methylxanthines had greater effect on tetanic contraction than on twitches of diaphragm muscle strips. Jones and colleagues (1982) however, reported the absolute increase in tension to be the same for both types of contraction, and Esau (1988) described increased force development at all frequencies of stimulation.

EFFECT ON FATIGUE

Skeletal muscle fatigue is also influenced by methylxanthines. Nassar-Gentina and colleagues (1981) found that caffeine reversed fatigue in single frog muscle fibres. However, there is disagreement concerning the concentration at which these changes are seen. Jones et al (1982), investigating a variety of animal and human muscle preparations, found that caffeine and aminophylline augmented skeletal muscle tension at low and high frequencies of stimulation and prevented low frequency fatigue.

DOSE-RELATIONSHIP

In vitro actions on muscle contraction have been studied mainly with caffeine and occur at low concentrations: 0.1-2 mmols/l. At higher levels, 5-10 mmols/l, muscle contracture occurs and at concentrations beyond this irreversible damage to skeletal muscle cells can occur (Luttgau and Oetliker 1968). Therapeutic concentrations of theophylline correspond to levels no higher than 0.5mmol/l, so that muscle contracture would not be expected to occur at usual in vivo blood levels.

EFFECT ON CONTRACTILE PROPERTIES

More than one component of skeletal muscle contraction is affected: speed of contraction is increased during both isotonic and isometric twitches (Sandow and Seaman 1964, Sandow and Preiser 1964), as is maximum rate of tension rise (Sandow et al 1965, Kentera & Varagic 1975). Theophylline increases the velocity of shortening at a range of loads during isometric contraction of diaphragm muscle strips (Supinski & Kelsen 1983).

EFFECT AT THE NEUROMUSCULAR JUNCTION

Esau (1986) found that theophylline increased the resting membrane potential in hamster diaphragm. There is no alteration in the shape of the muscle action potential, indicating that electrical activation of the muscle is unchanged (Sandow et al 1964).

Despite this, caffeine and theophylline are both known to stimulate neurotransmitter release from the nerve ending, and caffeine appears to enhance transmitter replenishment (Hofmann

1969, Wilson 1974). Thus methylxanthines may protect against high as well as low frequency fatigue, and cause greater amounts of neurotransmitter to be released for the same neural activation.

EVIDENCE FOR EFFECT AT THERAPEUTIC CONCENTRATIONS

Comparing the inotropic effect of theophylline on the isometric twitch and 20Hz tetanus of tracheal smooth muscle and diaphragm skeletal muscle strips, Supinski et al (1986) found that airway muscle responded at much lower doses than did the diaphragm.

Jones and colleagues (1982) concluded that the concentrations required for inotropic effects on skeletal muscle in vivo were likely to exceed the therapeutic spectrum. These findings were confirmed by Shee et al (1983) in studies on rat diaphragm. Although they found partial reversal of fatigue in rat diaphragm at concentrations of 0.1mM and beyond, they emphasized that such levels correspond in man to concentrations of more than 40mg/l, well above the therapeutic range in man. Derom and others (1990) confirmed this by showing that theophylline enhanced low frequency contractile force of canine diaphragm muscle bundles only at concentrations above those therapeutic in man.

In contrast, Kolbeck & Speir (1989), using a rib cage-diaphragm preparation, reported a 15% enhancement in diaphragm contraction at concentrations of theophylline corresponding to the upper limit of the therapeutic range in humans. Viires and coworkers (1986) employed a novel single diaphragm fibre model

to study effects, and maintained that increased contraction occurs at drug levels equivalent to *in vivo* therapeutic concentrations. It is unlikely, however, that the stated advantage of this model, in which all the muscle under study is accessible to drug, over the traditional *in vitro* model, really mimics the situation *in vivo*, where the majority of muscle fibres are exposed only to that level of drug which is transported via the blood-stream or through other muscle fibres.

IN VIVO ANIMAL STUDIES

EARLY WORK

Many workers have reported direct effects on skeletal muscle *in vivo*. In two studies, Huidobro (1945) and Huidobro & Amenbar (1945) investigated the action of theophylline, aminophylline, theobromine and caffeine on the quadriceps femoris muscle of anaesthetized cats. They found that all four methylxanthines increased the tension of indirectly stimulated muscle. The concentrations used were high (3-5% solutions). The effect at frequencies of stimulation up to 2.5Hz was slight and unreliable, whereas the potentiation of tension was greater at frequencies beyond this up to 20Hz. With still higher frequencies, to 250Hz, the action of caffeine was unremarkable (Huidobro & Amenbar 1945).

EFFECT ON CONTRACTILE PROPERTIES

Peak tension is increased, and the rate of relaxation is not affected (Howell et al 1981).

In fatigued muscle, aminophylline increases peak twitch tension, but does not reverse the reduction in speed of contraction, or the prolongation of half relaxation time caused by fatigue (Howell & Roussos 1984).

Hypercapnia also depresses peak twitch tension, an action reversed by aminophylline (Howell et al 1985). In addition, aminophylline increases time taken to reach peak contraction during hypercapnia.

DOSE-RELATIONSHIP

Di Marco et al (1982) noted that aminophylline improved the contractility of the stimulated diaphragm in anaesthetized dogs. This effect was apparent at blood concentrations of 10-20mg/l, the therapeutic range in man, and was greater at 40Hz than at 20Hz.

The influence of caffeine on transdiaphragmatic pressure development and on the total electrical response by the diaphragm, was studied in anaesthetized dogs during inspiratory efforts by Sigrist et al (1982). Transdiaphragmatic pressure improved for any given amplitude of electrical response, implying a direct effect on muscle contractility. The increase was dose-dependant. Significant effects were observed at doses starting at 20mg/kg yielding blood concentrations above 20mg/l, the upper limit of the therapeutic range in man.

The authors emphasized that during most studies, electrical output of the diaphragm after aminophylline was greater than during the control period. Despite this, only those results up to maximal control levels of electrical output were analysed.

Thus, the effect on all levels of transdiaphragmatic pressure relative to the electrical response was not determined.

Another factor which influenced the interpretation of the findings (Sigrist et al 1982), was that the configuration of the diaphragm was not strictly comparable between control and aminophylline studies. Improved force development may have arisen by alteration of diaphragmatic length and/or configuration to favour force development, as dictated by the length-tension relationship and by La Place's Law.

The influence of these factors and the unquantified effect of intercostal muscle contraction on pleural pressure development, and consequently on transdiaphragmatic pressure, make the interpretation of _in vivo_ studies difficult.

Derom and colleagues (1990) identified an inotropic effect on canine diaphragmatic contraction _in vitro_, but this occurred at very high concentrations. _In vivo_, in dogs they were unable to detect increased contractility or protection from fatigue at "attainable concentrations".

EFFECT IN LUNG DISEASE

The effect of aminophylline on muscle contraction is enhanced in the presence of hypoxia. Supinski and co-workers (1984b) found that in anaesthetized, mechanically ventilated dogs receiving phrenic nerve stimulation, hypoxia and hyperinflation decreased transdiaphragmatic pressure by 20% and 35% respectively, while aminophylline increased transdiaphragmatic pressure above normoxic levels. There were no associated changes in the extent of muscle activation as

judged from the compound muscle action potential, nor in blood pressure, pH, pCO_2, lung volume or chest wall dimensions as measured by inductance plethysmography.

Similarly, hypercapnia resulted in reduced peak twitch tension in anaesthetised dogs, affecting transdiaphragmatic pressure most at high frequencies of stimulation (Howell et al 1985). Aminophylline restored tension at all frequencies at both 20mg/kg and 40mg/kg concentrations. In contrast to its effect under normocapnic conditions, the half-time of muscle relaxation during hypercapnia was reduced with aminophylline, an action which might reduce force by increasing the frequency at which tetanus occurs.

EVIDENCE FOR THE SITE OF ACTION

After muscle denervation, contraction of the directly stimulated muscle is enhanced by caffeine at all frequencies of stimulation (Huidobro & Amenbar 1945). However, indirect stimulation produces a greater response than direct. The sensitivity of denervated muscle to acetylcholine is enhanced at low frequencies of stimulation. With administration of curare, there is reduction in contractile force which is partially reversed with methylxanthines. These effects were attributed primarily to lowering of the excitatory threshold of acetylcholine at the neuromuscular junction but also, in part, to a direct muscle effect. Breckenridge et al (1967), however, believed that enhancement of feline quadriceps femoris contraction with aminophylline was explained by an action at the neuromuscular junction.

To determine aminophylline's site of action, Aubier et al (1983a) studied ventilation in anaesthetized but spontaneously breathing dogs. $Pdi_{0.1}$, the transdiaphragmatic pressure and $Pt_{0.1}$, the tracheal pressure generated 0.1 second after airway occlusion, were used as indices of neuromuscular output to the diaphragm alone and to all the inspiratory muscles respectively, during spontaneous respiration. Ventilation, $Pdi_{0.1}$ and transdiaphragmatic pressure developed during stimulation at a range of frequencies all increased for any given electrical output of the diaphragm with administration of aminophylline, suggesting that the neural output of the phrenic nerve was not affected, while the response of the diaphragm was. The authors concluded that the drug's action to enhance ventilation arose from increased inspiratory muscle contraction rather than from central stimulation.

IN VIVO STUDIES - NORMAL SUBJECTS

EFFECT ON VOLUNTARY CONTRACTION OF THE RESPIRATORY MUSCLES

Aubier and colleagues (1981a) were the first to look into the effect of aminophylline on the human diaphragm in vivo. They studied voluntary inspiratory muscle strength in eight normal subjects before and during aminophylline infusion. A reproducible linear relationship was found between total electrical output of the diaphragm and transdiaphragmatic pressure developed on diaphragmatic contraction. Aminophylline tended to shift this relationship upwards, so that for a given electrical response, transdiaphragmatic pressure increased. A 15% increment in muscle force was found with aminophylline.

As the length of the diaphragm has a critical influence on pressure development, the abdomen was bound in order to oppose diaphragmatic descent, thereby keeping diaphragmatic configuration relatively constant. Diaphragmatic shortening occurs even under these circumstances however, so that the contraction can not be considered to be truly isometric (Loring et al 1985).

Supinski and colleagues (1984a) compared the action of caffeine on voluntary diaphragmatic contractility in normal subjects with that of aminophylline. Static inspiratory efforts were performed, and transdiaphragmatic pressure related to electrical output. At any given level of electrical output, transdiaphragmatic pressure was greater in all six subjects after caffeine and in five subjects after aminophylline.

EFFECT ON THE STIMULATED DIAPHRAGM

In vitro, methylxanthines are thought to have greatest effect on twitch tension. However in man this effect has not been confirmed. Moxham and co-workers (1985) studied diaphragmatic twitch tension produced by stimulation of one phrenic nerve in the neck. The unilateral diaphragmatic twitch did not change with therapeutic concentrations of theophylline. The authors of this study pointed out that an improvement of less than 10% may have been missed due to the fact that unilateral phrenic nerve stimulation was used. In addition, it is possible that the chest wall distortion occasioned by activation of one hemidiaphragm (Bellemare et al 1986) resulted in the dissipation of an increment in twitch tension.

An investigation without these disadvantages was carried out on effects of enprofylline, a bronchodilating methylxanthine and weak adenosine antagonist, and theophylline on the bilateral diaphragmatic twitch produced with needle electrodes in 10 normal subjects (Murciano et al 1987).

The size of the diaphragmatic twitch was considerably smaller than seen in previous studies in their laboratory, however the authors stated that this was due to a measurement difference between this and prior work. Another possibility is that the phrenic nerves were not maximally stimulated. The implication of this is that twitch tension might have increased merely because of greater phrenic nerve activation, rather than because of a true effect on the muscles by aminophylline. In fact, transdiaphragmatic pressure increased by 23% without concomitant changes in the compound muscle action potentials recorded from each hemidiaphragm. This indicates that electrical activation of the the diaphragm was very similar before and after administration of aminophylline.

Transpulmonary pressure, rib cage and abdominal dimensions were monitored to ensure constancy of lung volume and diaphragm configuration. The chest wall dimensions were measured by inductance plethysmography which records cross-sectional area rather than a single diameter and, although useful for monitoring lung volume, may not detect subtle alterations in chest wall diameters and hence diaphragmatic length (Grassino et al 1978, Loring et al 1985).

A more recent investigation of the effect of intravenous aminophylline on the bilateral diaphragmatic twitch

superimposed on a relaxed and contracting diaphragm failed to confirm these results (Levy et al 1990). In this study both inductance plethysmography and linearised magnetometry were used to monitor chest wall dimensions. Lung volume was measured in some of the subjects before and after aminophylline to ascertain whether a change occurred with the drug. There was no evidence of an alteration in these subjects. In order to reduce diaphragmatic shortening and thereby increase contractile force, subjects wore an abdominal binder. This had the effect of artificially increasing gastric pressure.

A small increase in the compound muscle action potential was seen after the drug, but this was not consistent between the two hemidiaphragms and there was no correlation between the size of the increment and serum theophylline level. Changes in twitch transdiaphragmatic pressure and the maximum transdiaphragmatic pressure achieved with performance of a modified Mueller manoeuvre were variable in any one individual on aminophylline. The authors concluded that the changes were thus unlikely to be due to an effect of aminophylline on phrenic nerve stimulation, on neuromuscular transmission or to a direct effect on muscle.

Aubier et al (1981a) investigated the diaphragmatic frequency-force relationship using unilateral phrenic nerve stimulation. This relationship is known to be reproducible in any individual and conforms to the same pattern for a wide variety of skeletal muscle (Edwards et al 1977a, Moxham et al 1980, 1981). After the subjects breathed through high

inspiratory resistances, the frequency-force curve was shifted down and to the right, with less pressure development at each frequency of stimulation. The result was both high and low frequency fatigue. The shift tended to be reversed after subsequent aminophylline (an improvement of approximately 15% being noted), and its severity to be lessened by prior administration of the drug.

A disadvantage with all the studies using phrenic nerve stimulation is that the studies are not conducted double-blind or with a placebo control. The former is unavoidable because of the easily recognisable effects of acute aminophylline administration.

STUDIES ON THE STERNOMASTOID

The sternomastoid is a respiratory muscle of particular importance to patients with airflow obstruction and hyperinflation (Skarvan & Milenkula 1970), and provides a simpler model for study than does the diaphragm. Two studies have investigated the effect of theophylline on the fresh and fatigued sternomastoid in normal subjects (Efthimiou et al 1986, Lewis et al 1986). Neither identified any increase in muscle strength of the muscle, nor an effect on the frequency-force relationship either before or after fatigue.

STUDIES OF VENTILATORY ENDURANCE

Ventilatory endurance has been measured as the time subjects are able to breathe through an inspiratory resistance, which is great enough to result in respiratory muscle fatigue (see Section 3, Page 64).

Supinski and co-workers (1984c) and Belman and colleagues (1985) both found that ventilatory endurance was prolonged with aminophylline. The former group reported that subjects experienced an alteration in the perceived magnitude of breathing effort required to maintain respiration through the inspiratory resistance, and believed that this effect was distinct from a direct action to improve respiratory muscle force (Supinski et al 1984c).

The latter workers found a small (2%) improvement in sustained ventilatory capacity with aminophylline, with no change in respiratory rate or tidal volume (Belman et al 1985). They postulated that their results could have been due either to increased neuromuscular excitability or to increased muscle contractility.

STUDIES DURING EXERCISE

It has been postulated that modification of exercise tolerance with methylxanthines in normal subjects and patients may relate to a direct action on skeletal muscle. This may be of particular relevance to COPD patients in whom respiratory muscle fatigue may limit exercise capacity (Grassino et al 1979).

Foltz et al (1943) studied the influence of aminophylline in both trained and untrained subjects performing rapidly exhausting work. They found no effect in untrained subjects. In trained subjects, the recovery from exhausting exercise was improved.

Athletes, exercising to exhaustion at 80% VO_2max, performed

longer after caffeinated than decaffeinated coffee (Costill et al 1978). Exercise was perceived to be easier with rather than without caffeine. This could be due either to a reduction in the effort required to perform the exercise or to central, psychological effects. Carbohydrate metabolism was identical during the two exercise periods, but lipid metabolism was enhanced with caffeine, resulting in less dependence on muscle glycogenolysis. This supports the view that a direct skeletal muscle effect was responsible for the findings.

In a similar study of healthy nonathletic men, Elliott et al (1985) found no difference between exercise with and without aminophylline. Heart rate at rest and at 50 watt increments in work rate was greater with the drug, although heart rate response to exercise was not altered.

STUDIES ON OTHER SKELETAL MUSCLES

Because of the complex geometric arrangement of the respiratory muscles around the chest wall, and the difficulty in making direct measurements of their force, other skeletal muscles have been used to study the action of methylxanthines on skeletal muscle in man. The anatomical relationship of the limb muscles to the joints on which they act, means that the force generated by contraction can be measured directly.

By virtue of its size and its simple lever action, the adductor pollicis provides a convenient model for study. Isometric manoeuvres can be made by stabilisation of the thumb and contraction against a rigid strap. The entire muscle can be completely activated through its motor nerve which is easily

accessible at the wrist, while the compound action potential can be recorded within the muscle.

Using this technique, Wiles and others (1983) documented frequency-force curves for the adductor pollicis in three healthy subjects. Fatigue was produced by intermittent voluntary contraction to a target set at 66% of the maximum voluntary contraction until the target could no longer be met. Frequency-force curves were recorded before and after fatigue, and were compared with those made with subjects on aminophylline. In addition, the maximal relaxation rate from contraction at 30Hz was measured before fatigue. Fatigue resulted in loss of force at low frequencies of stimulation. There was no change in the frequency-force relationship, nor was there resolution of low frequency fatigue with therapeutic concentrations of intravenous aminophylline.

In contrast, Lopes et al (1983) found that, with caffeine, the frequency-force relationship of the adductor pollicis was shifted upwards, with proportionately greater force development at low frequencies of stimulation. After fatigue, caffeine enhanced force at low frequencies with no change at high frequencies. Despite this, the authors found no increase in voluntary endurance during sustained voluntary contraction to a level of 50% of maximum. They concluded that caffeine had no effect on fatigue, but that the drug did improve muscle contraction.

RELATIVE EFFECTS OF CAFFEINE AND DIMETHYLXANTHINES

The two studies on the adductor pollicis (Wiles et al 1983,

Lopes et al 1983), and one by Supinski et al (1984a, see above) indicate a difference in the relative potencies of aminophylline and caffeine for effects on skeletal muscle contraction *in vivo*. This difference is borne out by *in vitro* work, and may explain why differing results have been found for the effects of theophylline at therapeutic concentrations. Some individuals may not be responsive to these doses.

STUDIES IN PATIENTS WITH COPD

There have been eight investigations of the effects of dimethylxanthines on the respiratory muscles in patients with COPD. Annother three studies have investigated walking distance and breathlessness.

Patients with COPD and airways obstruction unresponsive to standard (400mcg) doses of salbutamol were studied after acute administration of aminophylline (Davidson et al 1984) and, in a further study, following two weeks of regular dosing (Cooper et al 1985b). With acute administration, patients were able to walk further than previously, although they noted increased breathlessness scores implying that they exerted themselves to a greater extent with than without the drug (Davidson et al 1984). There were no changes in global respiratory muscle strength as measured by maximal static mouth pressures, nor in maximal ventilation or oxygen uptake during exercise. Ventilatory response to CO_2 rebreathing did show an increase, in keeping with previous work, suggesting in the light of the other findings that a central stimulatory effect was operating.

Following chronic administration, there were no changes in

walking distance, breathlessness scores during exercise, or in respiratory muscle strength (Cooper et al 1985b). Despite the fact that the patients were thought to have irreversible disease on entry to this study, significant bronchodilatation was achieved during the aminophylline period compared with that of the placebo period, although the changes were not significant when compared with the control phase of the study.

Belman and co-workers (1985) studied seven normoxic patients with COPD to determine whether increased diaphragmatic contractility resulted in enhanced ventilatory endurance measured as maximal sustained ventilation. The latter was 6.7% higher during an infusion of aminophylline, while there were no changes in respiratory rate, tidal volume or airway calibre. The authors felt that this response may have been due either to increased neuromuscular excitability or to a direct enhancement of respiratory muscle contractility. In addition, as ventilatory endurance is influenced by airway resistance as well as diaphragmatic strength, a reduction in resistance with aminophylline would lead to improved ventilatory endurance.

In contrast to this work, another study found that, with aminophylline, patients with COPD had increased maximum oxygen uptake with exercise, exercise duration, maximum work rate and maximal inspiratory mouth pressures (Nietrzeba et al 1984). The authors found no bronchodilatation, no increase in heart rate, maximum ventilation or oxygen uptake at incremental work rates, nor any alteration in PO_2, PCO_2 or pH at maximal exercise. The dyspnoea score with exercise showed a decrement

on the drug. This study was conducted without a placebo limb and analysed by comparing the control and aminophylline periods, so that awareness of the medication and learning effects may have appreciably influenced the results.

Foxworth et al (1988) investigated the dose relationship of intravenously administered aminophylline on the transdiaphragmatic pressure developed with maximal inspiratory efforts. This study followed a placebo-controlled, double-blind, randomised design with each of the 16 patients with COPD acting as his own control. Mean plasma concentrations of theophylline were 5, 12 and 19 micrograms/ml, and the active drug or dextrose solution used as placebo were each administered on a single day. Patients had only mild-moderate disease, in contrast to those entered to the studies by Murciano and co-workers (1984) and Belman and colleagues (1985).

No effect on transdiaphragmatic pressure was seen, and there was no evidence of a dose relationship. It is possible that had a measurement of ventilatory endurance or respiratory muscle fatigue been made, that a drug effect may have been found.

Hypercapnic and hypoxic COPD patients were studied by Murciano and colleagues (1984), in a 30 day single-blind study with 13 patients randomised to receive theophylline and five to placebo. The subjects were asked to breathe through inspiratory resistances until exhaustion, and the strength of the diaphragm was assessed from the performance of modified Mueller

manoeuvres. Fatigue was assessed from the high:low ratio of the diaphragmatic electromyogram recorded by oesophageal electrode. There were substantial changes in parameters of airway obstruction ie. forced expiratory volume in 1 second and forced vital capacity after 30 days of theophylline treatment, but no significant changes in functional residual capacity, maximum mid-expiratory flow or airway resistance. No alterations in any of these variables were found in the placebo group. Maximum transdiaphragmatic pressure increased on theophylline but not placebo. In addition, in those patients on active drug there were no changes in the high:low ratio when breathing through an equivalent inspiratory resistance to that at the start of the study.

This study was not double-blind, and the results may have been biased by knowledge of the prescribed medication. It was a parallel group design, and hence the small number on placebo might have reduced the chances of seeing an effect in this group (Murciano et al 1984). Fatigue was not shown to occur, because the high:low ratio of the electromyogram is not believed to give evidence that fatigue has occurred, only that it may be imminent, and it gives no indication of the timing of fatigue relative to the alteration in the ratio.

Although the modified Mueller manoeuvre has been advocated as a means of obtaining maximal diaphragmatic activation, it does not necessarily give the best indication of the inspiratory capacity of the diaphragm (Gibson et al 1981). In addition, it is difficult to perform; there was no indication in this report whether or not patients had had an adequate

learning period in which to become proficient at the test, nor the variability of the measurement. A learning period has been shown to be necessary for the comparatively more straighforward maximal static inspiratory manoeuvre measured at the mouth (Brophy et al 1985a). It is possible, therefore, that the effect seen was due to training or to learning, or to a general improvement in the clinical condition of the patients (Murciano et al 1984).

The presentation of the functional residual capacity values suggests that, although there were no significant changes, there did appear to be substantial (500ml) differences between pre- and post-aminophylline values, and this may have produced important differences in the lung volumes at which maximal transdiaphragmatic pressure and fatiguing inspirations were performed (Murciano et al 1984).

Kongragunta and colleagues (1988) also administered theophylline to moderately severe COPD patients. In this double-blind cross-over study, the drug was given for only three days prior to measurements being made, which, particularly as there was no placebo limb, may have resulted in subjects being aware of their treatment. Patients performed exercise and inspiratory loaded breathing to produce respiratory muscle fatigue, which was gauged by a fall in transdiaphragmatic pressure. There was no relationship between theophylline blood levels and changes in maximal transdiaphragmatic pressure, and no improvement in mean maximal transdiaphragmatic pressure with the drug.

Diaphragmatic electromyogram was measured via an oesophageal electrode during loaded breathing and exercise, and the high:low ratio and ratio of transdiaphragmatic pressure to electrical output of the diaphragm (Pdi:Edi ratio) were analysed (Kongragunta et al 1988). There were variable changes in the high:low ratio while the Pdi:Edi ratio showed more consistent falls with exercise. The authors felt that this probably did not indicate the production of respiratory muscle fatigue because the absolute transdiaphragmatic pressure actually increased from the beginning to the end of exercise, as did the electrical response of the diaphragm. This is in keeping with current knowledge of central nervous system output which appears to adapt to the effort required, possibly to ensure that muscle fatigue does not occur (Moxham 1990). The authors postulated that hypoxia or hypercapnia may be necessary before theophylline will produce an improvement in diaphragmatic contractility (Kongragunta et al 1988).

The changes in transdiaphragmatic pressure and electrical output were more substantial during loaded breathing with both falling by the end of the runs. However, there were no differences between the measurements on placebo and theophylline for the group (Kongragunta et al 1988).

A recent study by Murciano and co-workers (1989) investigated 60 patients with irreversible airways obstruction following a double-blind randomised design, with 8-week treatment periods on theophylline and placebo separated by an 8-day washout phase. As well as maximal inspiratory mouth pressures at functional residual capacity, the ratio of pleural

pressure during tidal breathing to the greatest pressure achieved during a maximal effort was measured. This is believed to be an index of respiratory muscle reserve (Roussos & Macklem 1977, De Troyer et al 1980a). In addition, breathlessness during "routine activity", functional residual capacity, airways resistance and flow-pressure curves were recorded.

Despite the fact that these patients had fixed airway disease, the mean forced expiratory volume in one second improved by 13% and mean forced vital capacity improved by 10% on active drug (Murciano et al 1989). There were no significant changes in airway resistance or in functional residual capacity. Mean pO_2 increased and pCO_2 decreased by 9% each. Minute ventilation and tidal volume increased by 19% and 17% respectively, while there was no change in respiratory rate.

The pleural pressure during inspiration did not alter on theophylline but did increase on placebo relative to the initial control period, while maximal transdiaphragmatic pressure increased by 22% on theophylline (Murciano et al 1989). The relative differences in the pleural pressure ratios thus reached 28%. Patients noted a decrease in their breathlessness scores on active drug with no change on placebo.

This large study produced results which are difficult to interpret. Patients had definite beneficial effects with theophylline, and although respiratory muscle strength increased, the study did not allow differentiation of the precise cause. Thus, as well as a direct effect on the respiratory muscles, central stimulation may have played a

part; in addition, the improvement in airway calibre may have assisted the development of higher inspiratory pressures on theophylline.

Another three studies have investigated subjective and objective parameters of disability (Eaton et al 1982, Evans 1984, Mahler et al 1985). In the first, patients received high and low-dose theophylline and placebo for one week period, at the end of which they performed 12 minute walks, cycle ergometry, pulmonary function tests and breathlessness assessment (Eaton et al 1982). No effect was found on either breathlessness or exercise tolerance although both doses of the active treatment resulted in increased forced lung volume measurements (Eaton et al 1982).

In the second study, single doses of theophylline were given to chronic bronchitic patients, and breathlessness, six minute walking distance and spirometry were measured (Evans 1984). No improvements in any parameter were seen.

In another double-blind, randomised, placebo-controlled trial, patients with moderately severe COPD received theophylline for four weeks (Mahler et al 1985). Breathlessness improved, while spirometry and 12 minute walk showed no change.

STUDIES IN QUADRIPLEGIC PATIENTS

Reports have described improvement in diaphragm muscle pressure development and in tidal volume with the administration of aminophylline to such subjects (Chevrolet et al 1983, Nochomovitz et al 1984). In the former, the primary

action appeared to be to enhance ventilation through a central effect. Transdiaphragmatic pressure did show an improvement, although the diaphragmatic electromyogram was not monitored so that this effect also may have been due to a central action.

In the study by Nochomovitz et al (1984) the patient underwent a training program to condition the paced diaphragm. The acute effect of aminophylline was assessed at the end of this period, and an improvement in transdiaphragmatic pressure was seen. The authors also noted that the increase in force did not always appear to be translated into a larger tidal volume. This may have been related to distortion of the compliant rib cage, or to variation in the force required to inflate the chest wall.

POSSIBLE MODES OF ACTION

The mechanism by which methylxanthines exert an inotropic effect on skeletal muscle is not yet established. There are differences both in the potencies of various methylxanthines for actions in different organ systems and the type of effects seen (Starr et el 1937, Boulanger et al 1982, Becker et al 1984, Howell & Fitzgerald 1985).

There are three possible mechanisms at the cellular level so that determining the one responsible for a single action has proven difficult:

1. Phosphodiesterase inhibition which results in increased cyclic AMP production, and thus greater availability of energy stores.

2. Adenosine receptor blockade which appears to occur at

lower methylxanthine concentrations than either mechanisms 1. or 3. (Sawynok & Jhamanadas 1976).

3. Alteration in intracellular/extracellular calcium concentrations (Nayler 1980, Aubier et al 1983a).

The latter mechanism has received greatest support. Calcium ions are essential for muscle glycogenolysis and for excitation-contraction coupling which results in muscle contraction (Sandow 1965). Bianchi (1961) showed that caffeine at high concentrations easily crosses skeletal muscle cell membranes, and causes marked release of calcium from muscle (Bianchi 1962). At levels (1mM) sufficient to cause twitch potentiation but not contracture, caffeine increased uptake and release of calcium (Isaacson & Sandow 1967). These workers found these effects to be dose-related.

Luttgau and Oetliker (1968) proposed the sarcotubular system as the site of drug action. Also in 1968, Weber and Herz reported that caffeine reduced calcium storage and rate of uptake by sarcoplasmic reticulum within skeletal muscle cells, and released sufficient calcium from reticulum to produce contracture. This was confirmed by Endo (1975).

It is thought that aminophylline may increase the flux of extracellular calcium through channels in the cell-membrane (Smith et al 1979, Varagic et al 1979). Extracellular calcium appears to be necessary for the action of theophylline but not of caffeine (Aubier 1983b, Esau 1988, Kolbeck & Speir 1989). This action itself may be mediated by adenosine receptor blockade, although Supinski and colleagues (1986) have shown that adenosine, while modifying the effect of theophylline on

airway smooth muscle, produces no alteration in effect of theophylline on the diaphragm.

Others have suggested that the effect of methylxanthines is brought about via release of adrenaline (Evans & Smith 1976). The actions of sympathomimetics and methylxanthines have many similarities and may have common pathways (Bowman & Nott 1969). The augmentation of skeletal muscle contraction by catecholamines increases with elevation of extracellular calcium ions (Montagu 1955), although catecholamine release itself is not dependant on the presence of extracellular calcium (Peach 1972).

Varagic and Zugic (1971) interpreted the enhancement of twitch tension with aminophylline as resulting from elevation of cyclic AMP levels in muscle. This could arise either from mechanisms 1. or 2. above. Phosphodiesterase inhibition is thought to act at the neuromuscular junction (Standaert et al 1976), but has been largely discredited as the cause of a direct muscle effect (Weber 1968, Kramer & Wells 1980). The inotropic effect of methylxanthines does not correlate with their relative potencies as phosphodiesterase inhibitors, or adenosine blockers (Kramer & Wells 1980, Jeppsson et al 1982, Persson et al 1982), nor with other important actions such as bronchodilatation (Daly 1982). On the other hand, it is possible that the apparent actions on muscle are, at least in part, due to an action at the neuromuscular junction; hence phosphodiesterase inhibition may be of importance to an indirect action on respiratory muscle.

SUMMARY

ACTIONS *IN VITRO* AND IN ANIMALS

Methylxanthines increase skeletal muscle tension at all frequencies of stimulation. Skeletal muscle fatigue, and particularly low frequency fatigue, is prevented or attenuated by methylxanthines.

Peak twitch tension, speed of contraction and the maximum rate of tension rise are increased. In fatigued muscle, aminophylline also increases peak twitch tension, but does not alter the fatigue-induced decrease in speed of contraction, or decrease in rate of relaxation.

Hypoxia causes an enhancement of effect, while the reduction in contractile force induced by hypercapnia is reversed.

Neuromuscular junction effects include reduction of the excitatory threshold of acetylcholine, and enhancement of neurotransmitter release and replenishment. However, *in vitro*, the shape of the action potential is not affected suggesting that electrical activation of the muscle is not altered.

Controversy exists about several of the inotropic effects. There is evidence that tension enhancement is greater with a twitch than with a tetanus, there are further data that the converse is true, and some results suggest that it is the same. The effects require relatively high concentrations of theophylline, and it is not clear whether significant effects are seen at therapeutic concentrations, although it is agreed that they appear to be dose-related.

EFFECTS IN MAN

In normal subjects, contractions during submaximal voluntary and stimulated inspiratory muscle contractions have been reported to increase by approximately 15% with therapeutic concentrations of aminophylline (Aubier et al 1981a, Supinski et al 1984a, Murciano et al 1987). In addition, fatigue was prevented and reversed after production with aminophylline (Aubier et al 1981a). Other work has found no effect on the diaphragmatic twitch (Moxham et al 1985, Levy et al 1990).

No effect has been found on the fresh and fatigued adductor pollicis and sternomastoid muscles of healthy volunteers at therapeutic concentrations (Wiles et al 1983, Efthimiou et al 1986, Lewis et al 1986).

Ventilatory endurance in normal subjects has been shown to increase to a negligible (2%) degree (Belman et al 1985). Exercise capacity was not altered in untrained subjects but the speed of recovery from exercise was improved in trained people (Foltz et al 1943), while exercise duration was extended in athletes (Costill et al 1978).

There have been eight studies on the respiratory muscles in patients with COPD. Four failed to identify an effect of theophylline on the strength of the respiratory muscles (Davidson et al 1984, Cooper et al 1985b, Kongragunta et al 1988, Foxworth et al 1988). Three studies showed increased inspiratory muscle strength (Murciano et al 1984, 1989, Nietrzeba et al 1984). The other study reported a modest (6.7%) increase in ventilatory endurance (Belman et al 1985).

Methylxanthines have been shown to prevent respiratory muscle fatigue (Murciano et al 1984), or to fail to affect it (Kongragunta et al 1988).

These contradictory results are in keeping with those from investigations of breathlessness and exercise tolerance; no effect being found on either parameter in three studies (Eaton et al 1982, Evans 1984, Cooper et al 1985b), a modest increase in six minute walk at the expense of increased dyspnoea in another (Davidson et al 1984), and an improvement in both breathlessness and walking distance in a fifth study (Mahler et al 1985).

There have been two case reports of an improvement in the contractility of the paced diaphragm (Chevrolet et al 1983, Nochomovitz et al 1984). In the first, the diaphragm was only partially denervated, and the authors suggested that aminophylline increased both central nervous system stimulation and muscle contractility (Chevrolet et al 1983). In the second study, the diaphragm was completely denervated, and muscle contractility improved with aminophylline (Nochomovitz et al 1984).

MODE OF ACTION

The mode of action is not yet established. The most likely cellular mechanism is enhancement of available intracellular calcium, possibly through adenosine blockade or via release of adrenaline from the adrenal medulla.

SECTION 5 - PREVIOUS METHODOLOGY

METHODS OF ASSESSMENT OF RESPIRATORY MUSCLE FUNCTION

There are a variety of methods of assessing respiratory muscle function *in vivo*. Because of the inaccessibility of the muscles, the complex geometry of the chest wall and the difficulty in separating their individual actions, the methods described have the disadvantage of being indirect, often qualitative and largely nonspecific for either the respiratory muscles as a system, or for any one respiratory muscle. In addition, precisely how respiratory muscle actions relate to function clinically is not yet fully determined: eg. the best way of demonstrating respiratory muscle fatigue has not been defined (Report of the Muscle Fatigue Workshop 1990).

As a consequence, different methods of investigation have been used and advocated by different workers, and this may account in part for many of the varying and sometimes contradictory results which have been reported.

HISTORY AND PHYSICAL EXAMINATION

Weakness of the respiratory muscles may be suspected from a history of dyspnoea on exertion, on lying flat, when going into deep water or swimming (Mier et al 1986) or at rest, of general tiredness, of morning headache or of difficulty in coughing. If weakness occurs in conjunction with other skeletal muscle dysfunction, the signs of the underlying disorder may be apparent to guide diagnosis; eg. muscle fasciculation in motor neurone disease.

Signs of respiratory muscle dysfunction include orthopnoea, tachypnoea, use of the accessory muscles of respiration, and abdominal paradox. These signs may occur late in disease, however, and particularly with weakness rather than paralysis, may not occur at all.

Symptoms and signs are of limited value as they provide only a qualitative guide to the presence of respiratory muscle dysfunction, and they may occur late in disease.

FLUOROSCOPY

The classical sign of diaphragmatic paralysis on diaphragmatic screening is paradoxical movement of the affected hemidiaphragm during a sniff (Alexander 1966). This sign is not reliable, while the more subtle sign of reduction in diaphragmatic excursion on respiration is even more difficult to judge (Alexander 1966). Bilateral paralysis may not be discernible due to extension of the vertebral column (Campbell & Newsom Davis 1970).

PULMONARY FUNCTION

A number of lung function tests depend on effort and skeletal respiratory muscle function, as well as on airway function, on the compliance of interstitial tissue and the chest wall, and on lung volumes. Interpretation of abnormalities, therefore, may not be straightforward.

In common with many other respiratory disorders, respiratory muscle weakness affects lung volumes, because of their dependence on effort. Total lung capacity and vital capacity are reduced, and residual volume is increased. The

position of functional residual capacity results from the balance of passive elastic forces in the lung and chest wall, the latter being influenced by respiratory muscle tone. Thus, functional residual capacity may be reduced in cases of neuromuscular disease affecting the respiratory muscles (De Troyer et al 1980b).

The simplest specific sign of weakness of the respiratory muscles is a fall in vital capacity of more than 25% on going from the seated to the supine posture, although patients with COPD may have a drop of as much 40% without diaphragm weakness (Allen et al 1985). Vital capacity measurements may not be affected in mild disease however, so that normal values do not necessarily exclude weakness (Black & Hyatt 1971, Demedts et al 1982). Serial measurements may be useful, as increases occur with successful treatment of respiratory muscle weakness (Mier et al 1988c & d).

CHEST WALL MOVEMENT

Konno and Mead (1967) discovered that during isovolume manoeuvres, volume changes occurred between the rib cage and abdomen and that these changes could be determined by monitoring rib cage and abdominal anteroposterior diameters. In addition, pulmonary ventilation can be quantified from chest wall movements (Goldman 1982). Abnormal patterns of rib cage and abdominal movement have been associated with pulmonary disease. Sharp et al (1980) found that paradoxical movement of the abdomen occurred during inspiration in patients with COPD. The same is true in diaphragmatic paralysis when the flaccid

diaphragm allows the transmission of negative pleural pressure to the abdominal cavity (Newsom Davis et al 1976).

The degree and direction of simultaneous movements in rib cage and abdominal anteroposterior dimensions are useful for the detection of paradox (Newsom Davis et al 1976, Sharp et al 1980) and more subtle and complex abnormalities of chest wall movement which are thought to occur with weakness or impending fatigue of the respiratory muscles (Cohen et al 1982).

The length and configuration of the diaphragm may alter without change in lung volume and, in keeping with Konno and Mead's work, such changes can be detected from simultaneous alterations in rib cage and abdominal anteroposterior diameters (Grassino et al 1978, Decramer et al 1985). This is not possible with measurement of lung volume alone (Grassino et al 1978, Loring et al 1985), because while a number of different diaphragmatic lengths can occur for the same lung volume, for given rib cage and abdominal diameters the diaphragmatic length is unique (Grassino et al 1978).

Some studies have utilised inductance plethysmography to monitor lung volumes (Murciano et al 1987). Changes in cross-sectional area can be recorded with respiratory inductance plethysmography, but the direction of movement of a single diameter cannot be determined by this technique. In addition, it is possible that the chest wall circumference might remain unchanged although two diameters may alter. Volume change may not be recognisable therefore. In addition, the technique cannot determine with certainty whether diaphragmatic length

and configuration have remained constant, and whether a measured change in transdiaphragmatic pressure is due to a change in contractility.

Measurement of chest wall diameters can be achieved with pairs of linearised magnetometers (Mead et al 1967). These consist of coils of wire. An electric current produced in one generates an electromagnetic field which is then detected by the partner coil. This, in turn, induces an electric current as output signal. The change in the induced current with movement of the coils away from and towards each other is inversely related to the cube of the distance between the two coils. The electrical output signal is linearised and calibrated to provide the measurement of distance between the pairs of magnetometers.

Use of the magnetometers has the advantage of being non-invasive and requiring no particular cooperation from the subject, and by detecting the displacement of rib cage and abdominal diameters, allows diaphragmatic length and configuration, as well as lung volume, to be monitored.

MAXIMAL STATIC MOUTH PRESSURES

The generation of pressure differentials between the airways and the atmosphere by the contraction of the respiratory muscles during respiration results in the flow of air into and out of the lung. Because of their inaccessibility and geometry, the force output of the respiratory muscles cannot be directly measured; however, the pressure generated within the thoracic cavity is freely transmitted along airways

to the mouth and can be measured. Global respiratory muscle strength can be assessed by measuring the pressure generated at the mouth during maximal inspiratory and expiratory efforts (Black & Hyatt 1969).

The pressures generated are volume-dependent (Agostoni & Campbell 1970). Maximal static expiratory pressures (PEmax) are performed at full lung expansion, so that if total lung capacity is reduced through lung disease eg. fibrosis or where weakness of the inspiratory muscles prevents the attainment of total lung capacity, PEmax may be underestimated. Maximal static inspiratory pressures (PImax) are performed from either functional residual capacity or residual volume. An increase in either volume, because of emphysema or weakness of the expiratory muscles, may result in underestimation of global inspiratory muscle strength.

The adequate performance of the pressures is also dependent on the subject's cooperation and effort and his ability to hold the mouthpiece in the mouth without a leak at the lips. In practice, patients find the maintenance of a seal around the mouthpiece to be easily possible. A small leak in the mouthpiece prevents the generation of falsely high pressures with the cheek muscles (Ringqvist 1966).

Some workers have found mouth pressures to be difficult for patients to perform and unreliable because of a substantial learning effect (Shephard et al 1958, Cook et al 1964). Careful instruction in the performance of maximal inspiratory and expiratory efforts over a short learning period ensures that the repeatability approaches that of other lung function tests

(Chapter 2, Section 3, Pages 160-162), (Brophy et al 1985a).

Comparison of results from different studies is hampered by the use of a variety of techniques for the generation of maximal inspiratory manoeuvres. As well as the relatively simple maximal inspiratory effort (Mueller manoeuvre) used in this thesis, the modified Mueller manoeuvre has been employed in many studies by other workers (Roussos & Macklem 1977). This technique was developed because the diaphragm has both an inspiratory and expulsive effect, and because full activation may not occur without the generation of high abdominal pressures. Although it often results in higher pressures than the Mueller manoeuvre, the modified Mueller manoeuvre has proven difficult to master in our laboratory. Patients have been unable to perform it consistently or reliably. To an even greater extent than the Mueller manoeuvre, it also may not reflect the behaviour of the diaphragm or inspiratory muscles during respiration, and so may be irrelevant to the interpretation of diaphragmatic function clinically (Gibson et al 1981). As a consequence the test has not been used in any of the studies subsequently described.

Inspiratory and expiratory mouth pressure measurements are helpful in the assessment of respiratory muscle function because they depend independently on the muscles serving ventilation and those responsible for cough and expectoration respectively. The chief disadvantage is that the normal ranges are wide with low minima, making recognition of weakness difficult (Black & Hyatt 1969, Wilson et al 1984a). Despite

this, because mouth pressure measurements are non-invasive, simple to perform and repeatable over time, they are very suitable for the serial monitoring of global inspiratory and expiratory muscle strength (McElvaney et al 1989). For these reasons, and because global muscle strength, rather than just diaphragmatic strength, is important in determining the susceptability of a patient to weakness or fatigue, mouth pressures have been used in the studies in this thesis.

TRANSDIAPHRAGMATIC PRESSURE (PDI)

The consequence of respiratory muscle contraction is the development of pressure within the abdominal and thoracic cavities. These pressures can be measured with fluid-filled balloon catheters, attached to differential pressure transducers and placed in the stomach and the oesophagus respectively (Agostoni & Rahn 1960, Milic-Emili et al 1964a).

Oesophageal and gastric balloon catheters measure oesophageal pressure (Poes) and gastric pressure (Pg) which have been shown to accurately reflect pleural and abdominal pressures (Milic-Emili et al 1964a, b & c, Agostoni & Rahn 1960) Their differential (Pdi=Pg-Poes) provides a reliable indication of the pressure generated by the diaphragm during contraction (Kim et al 1976).

During the inspiratory phase of tidal breathing and vital capacity manoeuvres, the diaphragm contracts and descends and Pdi increases, due to a decrease in Poes and an increase in Pg. If the diaphragm is paralysed, no pressure differential is detectable and zero Pdi is recorded. In cases of weakness, the

Pdi generated during tidal breathing and vital capacity manoeuvres may be small, but still normal. Because the normal ranges are wide, with very low minimum values (De Troyer & Estenne 1981), the utility of these manoeuvres in the detection of diaphragmatic weakness is poor. Nevertheless, it may give an indication of weakness when used in conjunction with other parameters (Mier et al 1988d).

The maximal inspiratory manoeuvres used during maximal inspiratory mouth pressure measurement (Mueller manoeuvre & modified Mueller manoeuvre) also can be utilised for measuring Pdi. Added to the problems associated with the performance of these maximal efforts during mouth pressure measurement (see above Page 115) is the distraction occasioned by the discomfort of the balloon catheters rubbing against the nasal mucosa. This may be severe enough to inhibit the satisfactory performance of the manoeuvres. In addition, in the case of the modified Mueller manoeuvre, less negative Poes results than with a Mueller manoeuvre (Gibson et al 1981), so that the Pdi generated may not accurately reflect its capacity as an inspiratory muscle.

An alternative more natural manoeuvre than the sustained maximal inspiratory efforts is a short sharp maximal sniff from resting end-expiration. The maximal sniff generates high pressures with a much greater lower limit of normal than the Mueller manoeuvre, modified Mueller manoeuvre or vital capacity (Miller et al 1985). The range and reproducibility of sniff Pdi has been assessed in our laboratory in normal subjects, and has a higher lower limit of normal than other tests of

diaphragmatic function (Miller et al 1985). In a study of 64 healthy volunteers (27 females), the range of sniff Pdi values was 82-204cms H_2O, with a mean and standard deviation for females of 121.5 ±25.2cms H_2O, and for men, values of 147.8 ±24.1cms H_2O. In addition, the sniff is simple to perform and highly reproducible in normal subjects (Miller et al 1985). Also, it sensitively detects abnormal respiratory muscle function and alteration with progress of disease or treatment (Laroche et al 1988b, Mier et al 1989).

Sniffs are performed from resting end-expiration, which in healthy subjects is at a stable lung volume. Thus, slight changes in the lung volume at which measurements are made have little effect on results, and the recorded respiratory pressures accurately reflect respiratory muscle strength.

PHRENIC NERVE STIMULATION

Each hemidiaphragm can be activated by stimulation of the ipsilateral phrenic nerve. This allows the central nervous system to be bypassed, the integrity of the nerve to be tested by measurement of nerve conduction time (Newsom Davis 1967, Loh et al 1977), and diaphragmatic contractions to be recorded under controlled conditions, all without having to rely on the effort and motivation of the subject.

MECHANICAL ACTIVITY OF THE STIMULATED DIAPHRAGM

Phrenic nerve stimulation can be applied to produce single twitches or tetanic contractions. The former provides information on muscle strength, as the amplitude of bilateral

twitch approximates to one fifth that of a maximum contraction (Aubier et al 1985c, Mier et al 1985d), and on the contractile properties of the muscle from the rates of muscle contraction and relaxation. Tetanic stimulation allows construction of a frequency-force curve, and identification of the production of and recovery from low and high frequency fatigue (see Chapter 1, Section 3, Page 63) (Edwards et al 1977b, Moxham et al 1980).

Although the unilateral frequency-force curve of the diaphragm has been studied (Aubier et al 1981a, Moxham et al 1981), unilateral stimulation results in distortion of the chest wall and the measured Pdi probably underestimates true diaphragmatic contraction (Bellemare et al 1986, Mier et al 1992).

Bilateral stimulation with both hemidiaphragms contracting simultaneously mimics true physiological diaphragmatic activation more closely than does unilateral stimulation (Bellemare et al 1986), with the result that the values for bilateral twitches are more than twice those for unilateral twitches (Mier et al 1985d, Bellemare et al 1986). However, it is difficult to achieve tetanic stimulation reproducibly even in highly motivated normal subjects (Levy et al 1990). This, together with the fact that it can be extremely uncomfortable makes bilateral tetanic stimulation unsuitable for use in patients.

Bilateral twitches are well tolerated by some patients however, and the recorded twitch Pdi is highly repeatable within a subject over several days (Mier et al 1985d).

Consequently, bilateral twitch Pdi has been used in cooperative patients for this thesis (Chapter 7).

As is the case with other types of diaphragmatic contraction, twitch Pdi increases with lung volumes below functional residual capacity and decreases as lung volume increases above functional residual capacity (Mier et al 1985c). It is important to ensure that lung volume and diaphragmatic configuration are similar for the twitches to be reliably comparable. In some studies of the effect of aminophylline on twitch Pdi (Moxham et al 1985, Murciano et al 1987), data on lung volume and chest wall dimensions do not appear to have been matched for the control and post-aminophylline study periods. In this thesis, the twitches have been matched as closely as possible, to allow comparison between treatment periods (Chapter 4).

ELECTRICAL ACTIVITY OF THE STIMULATED DIAPHRAGM

As well as Pdi, the electrical activity of the diaphragm (the electromyogram [EMG]) also can be recorded during artificial phrenic nerve stimulation, by means of surface electrodes or an oesophageal probe. Surface electrodes record the signal from each hemidiaphragm independently, the electrodes are non-invasive and simple to attach. The chief disadvantage is that they pick up the electrical activity of underlying intercostal or abdominal muscle fibres in addition to that of the diaphragm.

The oesophageal electrode gives a sensitive signal uncontaminated by other muscles; however, it records only from

the crural and not the costal diaphragm, and it does not differentiate between the contraction of the two hemidiaphragms; hence adequate bilateral activation cannot be reliably identified. The oesophageal electrode is also invasive, necessitating the swallowing of a thick wire, which makes it unacceptable to many subjects and patients, even when oesophageal and gastric pressure balloons are being used.

Surface electrodes have been used in this work. As they have been employed during artificial stimulation of the diaphragm with subjects seated at rest, the problem of contamination by the activity of other muscles has not arisen.

The EMG is critically dependent on constancy of diaphragmatic length and configuration, lung volume and the state of contraction of other chest wall muscles (Grassino et al 1978, Gandevia & McKenzie 1986). Thus, although the amplitude of the EMG provides information on the degree of activation of the ipsilateral hemidiaphragm, it is influenced by other factors which must be taken into account in the interpretation of results.

PDI:EDI RATIO

The EMG signal can be rectified and integrated so that a quantified signal is obtained. Some workers have summed the EMG amplitudes from each side and related the result to the mechanical output of the diaphragm to obtain the Pdi:Edi ratio (Edi - electrical output of the diaphragm), citing changes as evidence of increased or decreased contraction (Aubier et al 1981a, Supinski et al 1984a).

Aubier and colleagues (1981a) and Supinski and co-workers (1984a) report that during voluntary contraction of the diaphragm when Edi increases, so too does Pdi, and that the ratio of the amplitudes of Edi to Pdi is stable within studies in a given subject. However, electromyograms recorded by either oesophageal or surface electrodes change artefactually in a variety of circumstances, eg. during positive pressure breathing (Green et al 1978), with changes in diaphragmatic muscle fibre length (Kim et al 1985), lung volume and posture (Gandevia & McKenzie 1986). Furthermore, the Edi might remain constant despite variation in each hemidiaphragm EMG, so that the Edi itself provides less information than do the left and right EMG amplitudes.

In addition, use of the ratio to determine the size of effect of theophylline on Pdi has depended on the assumption that the relationship between them is linear, when early work has shown it to be curvilinear under controlled, isovolumetric and isometric conditions (Grassino et al 1978). For these reasons, the practice has not been adopted here (Study 4).

The Pdi:Edi ratio has been used to study voluntary static contractions (Grassino et al 1978), although EMG recordings during such efforts are subject to artefact (Gandevia & McKenzie 1986). It may be even less applicable to stimulated contractions (Levy et al 1990), when the diaphragm is the sole contracting muscle and chest wall distortion occurs.

Recent work has shown that, even if maximal activation of both hemidiaphragms is not effected during bilateral phrenic nerve stimulation, a maximal diaphragmatic contraction can

still result, as long as one hemidiaphragm is maximally activated and the other contracts sufficiently to prevent the chest wall distortion that will otherwise occur (Bellemare et al 1986, Nava et al 1987). Thus a failure of complete phrenic nerve stimulation on one side with reduction in the EMG may not necessarily imply failure of ipsilateral diaphragmatic activation, nor a falsely low twitch Pdi.

In the study presented here (Chapter 4), an attempt has been made to compare twitches which have similar right and left EMG amplitudes, and which have been produced at similar rib cage and abdominal dimensions, so that factors known to influence twitch Pdi are controlled as far as possible.

ELECTROMYOGRAM (EMG) POWER SPECTRUM

The frequency spectrum of the EMG can be analysed and a ratio of the high and low frequency components obtained (Moxham et al 1982). A fall in this ratio has been shown to precede the development of diaphragmatic fatigue, so that it is a predictor of fatigue should the same or a greater level of contraction continue, but not evidence that fatigue has occurred (Bellemare & Grassino 1982b). The only certain way of identifying fatigue-development is by demonstrating a fall in Pdi but without change in lung volume, diaphragmatic length and configuration or left and right EMG.

MODEL OF RESPIRATORY MUSCLE FUNCTION - THE QUADRICEPS FEMORIS

The morphology and physiology of respiratory muscles appear typical of other skeletal muscles. Nevertheless, the diaphragm is unique because it is required to contract repeatedly without

long periods of rest. In vivo recording of force and electrical output from human respiratory muscles is hampered by their inaccessibility however, while the number, complex geometry and interrelationship of the respiratory muscles make the effects on the respiratory system of changes in individual muscles difficult to interpret. For these reasons, research workers have used other skeletal muscles, such as the adductor pollicis and the sternomastoid as models of respiratory muscle function (Wiles et al 1983, Lopes et al 1983, Efthimiou et al 1986, Lewis et al 1986).

One other model is provided by the quadriceps femoris which, like the diaphragm, is a large muscle required to maintain tone and to function over long periods of time. Unlike the diaphragm, it is accessible, it can be directly stimulated and its action can be directly and easily measured, so that it provides a practical means of studying the action of drugs in man, utilising techniques which cannot be used reliably on the respiratory muscles (Edwards 1977a). Disadvantages are that it does not function continuously and its muscle fibre composition differs slightly from that of the diaphragm.

SUMMARY

There are a variety of indirect means of investigating respiratory muscle function. History, examination, radiological screening of the diaphragm and lung volume measurements are all non-specific and qualitative methods.

Other more specific techniques include monitoring rib cage and abdominal diameters with linearised magnetometers, which

allow muscle contractility to be compared during periods of constant diaphragmatic configuration and length.

A more quantitative test is maximal static mouth pressures, which have a wide normal range, but are valuable for repeated recording of global inspiratory and expiratory muscle strength.

Transdiaphragmatic pressure quantifies diaphragmatic contraction. Pressures measured on breathing to total lung capacity and the Mueller and modified Mueller manoeuvres, have wide normal ranges and very low normal minima, while the normal maximal sniff has a narrower range and higher minimum value, thereby allowing recognition of weakness and fatigue.

Phrenic nerve stimulation allows the diaphragm to be studied without the need for effort on the part of the subject. Bilateral twitches produce a more normal physiological contraction than unilateral twitches, because distortion of the chest wall and contralateral hemidiaphragm occurs with unilateral stimulation.

SECTION 6 - OBJECTIVES

It is unclear from current work whether the inotropic effect of dimethylxanthines on skeletal muscle occurs at therapeutic concentrations, what is the magnitude of effect *in vivo* in man, and whether both maximal and submaximal contractions are affected. The clinical relevance of any action has also not been determined.

In order to clarify the action of dimethylxanthines on skeletal muscle and respiratory muscle function five separate studies were performed. They had the following objectives.

1. To determine the effect of chronic oral theophylline at therapeutic concentrations on the strength and frequency-force relationship of the quadriceps femoris before and after the production of low frequency fatigue, in normal subjects.

2. To study the effect of therapeutic concentrations of intravenous aminophylline on the bilateral diaphragmatic twitch in normal subjects.

3. To investigate the effect of intravenous aminophylline at therapeutic concentrations on the paced diaphragm of quadriplegic patients, by measuring transdiaphragmatic pressure, tidal volume and diaphragmatic electromyogram.

4. To study the effect of therapeutic concentrations of chronic oral aminophylline on respiratory muscle strength and quadriceps muscle strength and endurance in normal subjects.

5. To investigate the effect of chronic oral theophylline and aminophylline on dyspnoea, respiratory muscle function, airways obstruction, lung volumes and quadriceps femoris strength in patients with COPD.

CHAPTER 2

GENERAL METHODOLOGY

SECTION 1 - SUBJECTS AND METHODS

The subjects, methods and equipment used in the five studies of this thesis are presented below. The methods and protocols unique to a single study are presented in the relevant chapter.

SUBJECTS

Healthy subjects were recruited from the medical staff of the Brompton Hospital and the Cardiothoracic Institute, London University. Patients in Study 5 were recruited from those attending physicians at the Brompton Hospital. All procedures and the protocol was passed by the Ethics Committee of the Brompton Hospital. The quadriplegic patients in Study 3, who were under the care of clinicians at King's College Hospital, were studied as part of their clinical assessment and the protocol was not submitted for Ethics Committee approval.

All subjects and patients had the purpose of the studies and the procedures explained, and gave their verbal informed consent to their participation in the investigations. All were informed that they could withdraw at any time from the studies and, in the case of the patients, that appropriate treatment of their condition would not be influenced by their participation, or not, in the work.

METHODS

The techniques used are all recognised methods of investigation of pulmonary function, the respiratory and quadriceps muscles, as detailed in Chapter 1, Section 5. Two

parameters of respiratory function assessment, the sniff and the diaphragmatic twitch, are available in a few specialised units. Prior to their use in the studies in this thesis, the repeatability of respiratory and quadriceps force measurements were assessed (Chapter 2, Section 3).

The technique of low frequency fatigue production used in Study 1 was based on a method developed by Edwards and colleagues (1977a), and used in studies by Wiles and co-workers (1983) on the adductor pollicis muscle.

LUNG VOLUMES

Tidal breathing and vital capacity were recorded during respiratory muscle studies with subjects seated and supine. Subjects wore a nose-clip and breathed into and out of a dry spirometer through a firm rubber flanged mouthpiece. Between each vital capacity, subjects rested for approximately one minute, and removed nose-clip and mouthpiece to breathe normally. Patients who felt breathless on lying flat performed volume measurements semi-recumbent with the bed-head at 20° head up tilt, and were helped to sit upright in between manoeuvres.

MAXIMAL STATIC MOUTH PRESSURES

Subjects were seated and breathed through apparatus consisting of a firm rubber flanged mouthpiece attached to a metal tube incorporating a small leak (Ringqvist 1966). The mouth-piece was held within the mouth. The leak prevented glottic closure and creation of pressure with the cheeks. The metal tube was connected to the spirometer in order to monitor

the lung volume at which the efforts were performed.

MAXIMAL STATIC EXPIRATORY MOUTH PRESSURE (PEMAX)

Subjects inhaled to total lung capacity, at which point a tap in the mouthpiece was turned to occlude the airway, except for the small leak. They were instructed to perform a maximal effort consisting of a sustained maximal expiration similar to the technique required to blow up a balloon.

MAXIMAL STATIC INSPIRATORY MOUTH PRESSURE (PIMAX)

Subjects performed tidal breathing, stopping at relaxed end-expiration, ie. functional residual capacity, or breathing out slowly and fully to residual volume. The airway was occluded and they were asked to perform a maximal inspiration in a similar way to sucking forcefully through a narrow bore straw.

Static inspiratory mouth pressure measurements are negative, but for convenience, throughout the thesis, a numerical decrease is referred to as an improvement or increase.

For each manoeuvre, subjects were asked to sustain the effort for 2-5 seconds. Enthusiastic encouragement was given by the investigator, and subjects were able to gauge their efforts from the storage cathode ray oscilloscope (CRO) trace. In order to prevent this negatively influencing subsequent efforts, the screen was cleared after each manoeuvre and the calibration was altered between visits. Three technically satisfactory measurements for each parameter were required, and this generally entailed between 3-6 efforts at each lung

volume. The pressure sustained for one second was taken as the maximal mouth pressure.

Chest wall configuration was not controlled during maximal respiratory efforts. However, maximal static expiratory and inspiratory mouth pressures vary little near total lung capacity or residual volume respectively (Agostoni & Rahn 1960), and by performing vital capacity immediately before each manoeuvre the constancy of lung volumes was checked.

TRANSDIAPHRAGMATIC PRESSURE (PDI)

Transdiaphragmatic pressure was measured with balloon catheters as described in Chapter 1, Section 5, Page 116, during phrenic nerve stimulation at 1Hz in Studies 2 & 5, phrenic nerve pacing in Study 3, maximal sniffs in Studies 4 and 5, and maximal inspiratory efforts and respiration in Study 5.

Prior to use, the balloon catheters were emptied of air by placing them at least five centimetres (cms) below water and opening them to atmosphere via a three-way tap. The tap was then turned so that catheters were closed to atmosphere but open to the transducers.

After spraying the nasal mucosa with 1% lignocaine spray the balloons, coated with 2% lignocaine gel, were advanced gently into the nose, until the subject became aware of them at the nasopharynx. At this point, subjects were asked to sip water through a straw and to swallow. In general, the catheters could be felt by the investigator being pulled proximally with each swallow. I performed the technique

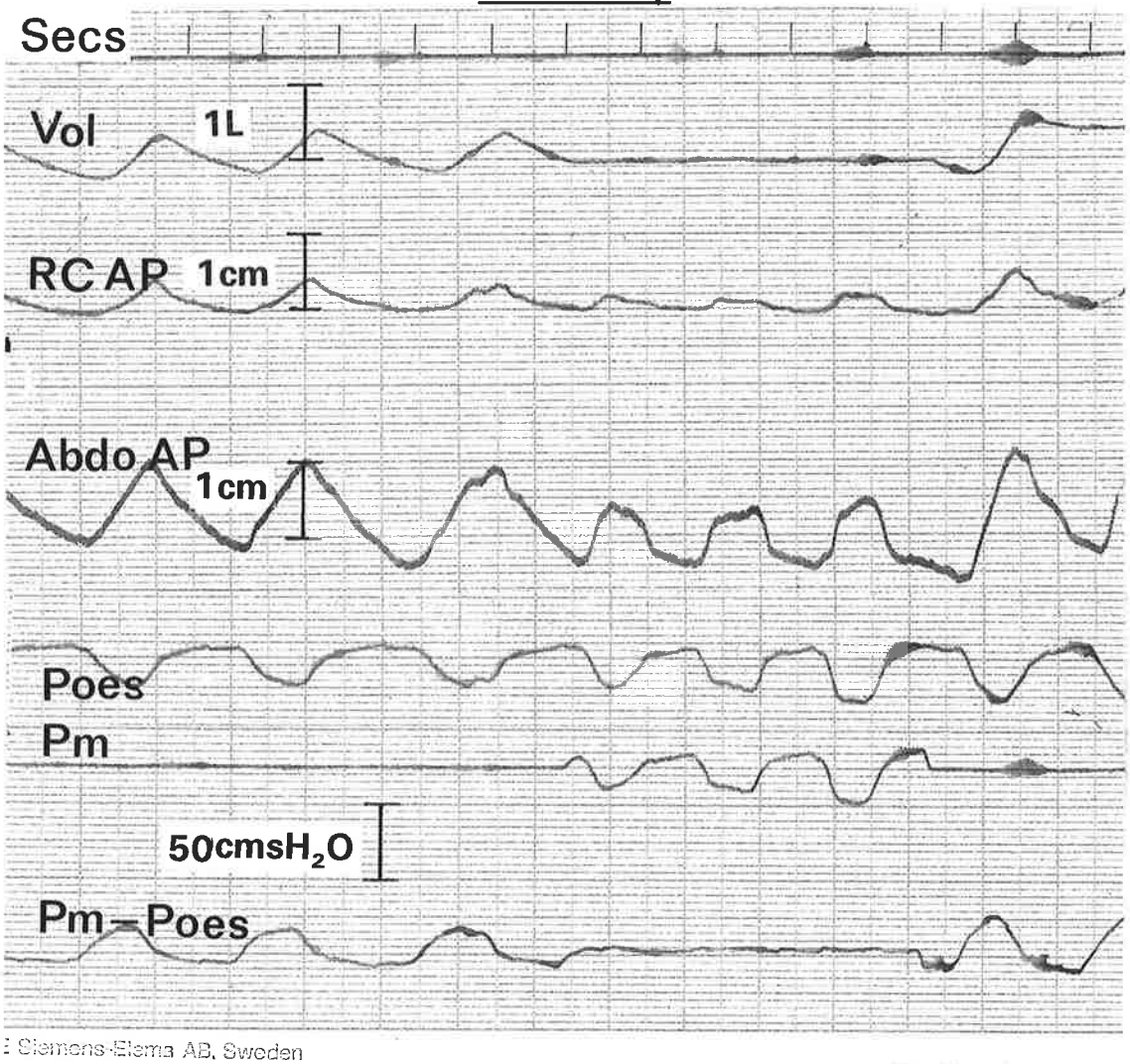
approximately 100 times and in only three or four cases was the subject unable to tolerate the procedure. In one individual the catheters were passed into the trachea, due to bulbar palsy and difficulty swallowing. This was recognised immediately because of cough and by the appearance of the pressure trace. The catheters were removed and passed again successfully.

When both catheters were passed so that the tips lay in the stomach, approximately 70cms from the nares, each was filled with five millilitres (mls) of air from a glass syringe. Three mls were removed from the gastric and 4.5mls from the oesophageal catheter. The latter was then slowly withdrawn to lie with the balloon tip between 40-45cms from the nares, so that it lay in the mid-third of the oesophagus (Milic-Emili et al 1964a & b). The catheters were then taped securely to the cheek with micropore adhesive tape.

With the oesophageal balloon at the correct level the resting pressure recorded by it is negative, reflecting the resting negative pleural pressure in the thorax (Milic-Emili et al 1964a). On inspiration, oesophageal pressure becomes more negative, while the gastric pressure becomes more positive.

Satisfactory positioning of the oesophageal balloon was checked by the performing a gentle respiratory effort against an occluded mouthpiece. This occlusion test (Baydur et al 1982) is based on the fact that the oesophageal catheter should record the same amplitude and shape of pressure trace as that recorded at the mouth which, with the glottis open, will accurately represent intra-thoracic pressure (Figure 2.1).

FIGURE 2.1



Siemens-Elema AB, Sweden

A trace of an occlusion test performed in a patient with COPD, showing from the top, the one second timer, volume (Vol), rib cage anteroposterior diameter (RC AP), abdominal anteroposterior diameter (Abdo AP), oesophageal pressure (Poes), mouth pressure (Pm), and the difference in pressure between mouth and oesophageal pressure traces (Pm-Poes). The shape and magnitude of pressures recorded at the mouth and in the oesophagus are the same after occlusion of the airway, indicating that the oesophageal balloon is correctly positioned.

INSPIRATION

Transdiaphragmatic pressure during tidal breathing and a full inspiration were measured in patients while sitting and supine to find evidence of alteration in respiratory muscle strength (Study 5). As detailed in Chapter 1, Section 5, Pages 116-118, Pdi during full inspiration provides limited quantitative information, however it may give an indication of respiratory dysfunction, when used with other measurements.

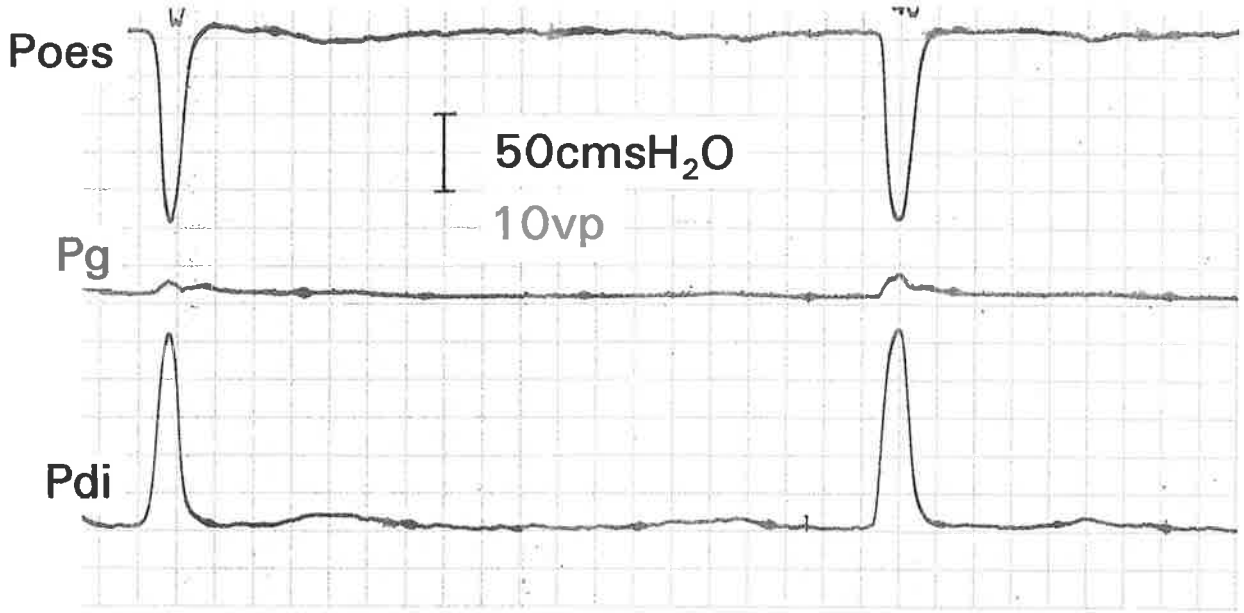
MAXIMAL INSPIRATORY EFFORT

De Troyer & Estenne (1981) have reported that the Pdi developed during the performance of a maximal inspiratory effort has too wide a normal range for use as a reliable indication of inspiratory muscle weakness. Despite this, recorded serially, the coefficient of variation allows comparison of inspiratory muscle strength in the same way as pressures recorded at the mouth (Miller et al 1985).

MAXIMAL SNIFF

Maximal sniffs were performed with subjects seated and without wearing a nose-clip. Subjects were asked to breath quietly and at the end of a relaxed expiration to perform a short but vigorous sniff (Miller et al 1985). Sniffs were displayed on the cathode ray oscilloscope to encourage effort. A series of 4-8 practice sniffs were performed after which a series of at least 10 maximal efforts were recorded. Examples of normal sniff traces are given in Figure 2.2.

FIGURE 2.2



A trace of oesophageal pressure (Poes) (top), gastric pressure (Pg) (middle) and transdiaphragmatic pressure (Pdi) (bottom) during two maximal sniffs, performed by a normal subject. During the manoeuvre Poes becomes negative, Pg becomes more positive, and Pdi, the difference between Pg and Poes (Pdi=Pg-Poes) becomes more positive.

PHRENIC NERVE STIMULATION

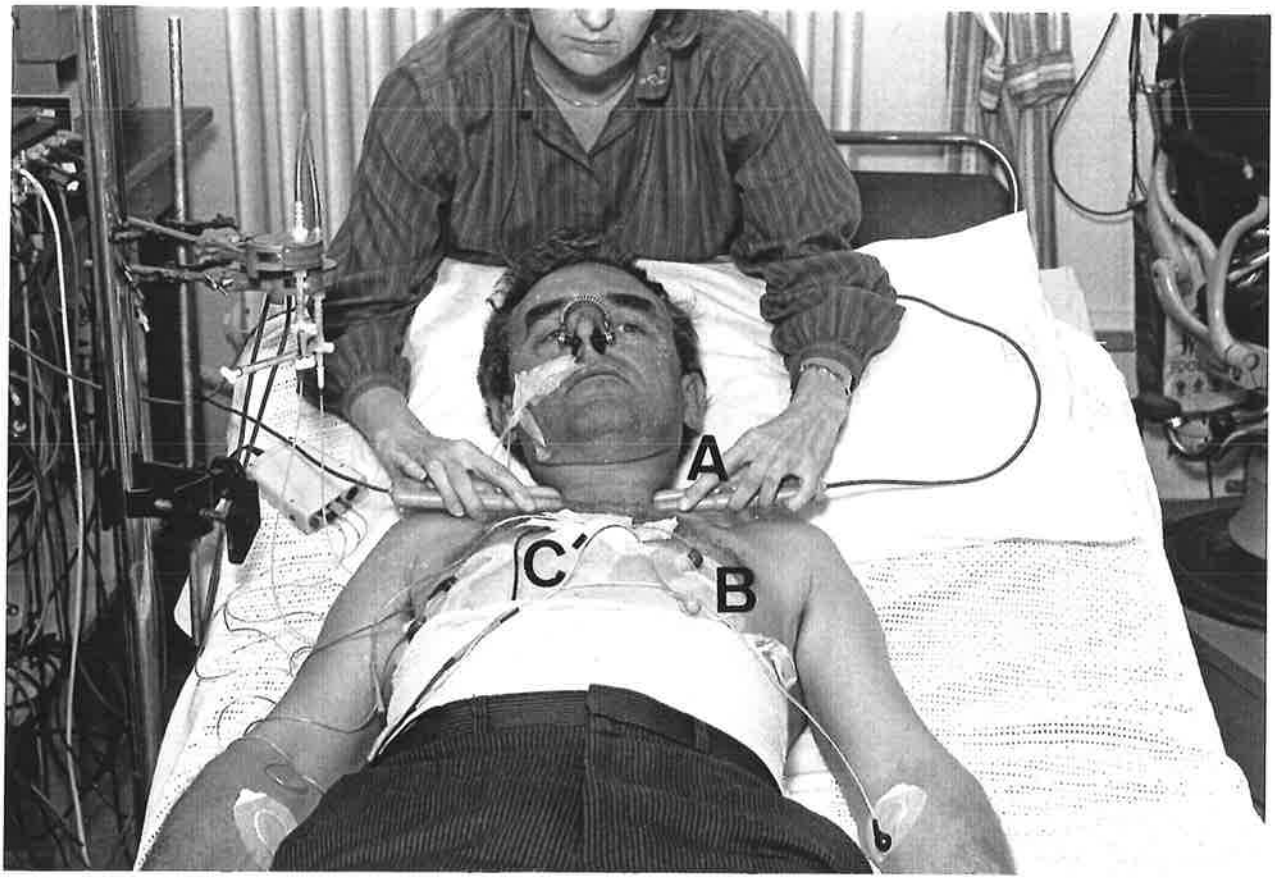
This technique has been used in patients at the Brompton Hospital to demonstrate the integrity of the phrenic nerve, and to investigate diaphragmatic contractility. The procedure is generally well-tolerated, producing a slight pricking sensation in the skin of the neck and a sensation of hiccoughs as the diaphragm contracts. The pricking feeling may become painful at high voltages, and during tetanic stimulation however.

Phrenic nerve stimulation was carried out with subjects relaxed and, in Study 2 (Chapter 4), seated upright in a chair. In Studies 3 and 5 (Chapters 5 & 7), patients were either supine or semi-recumbent at 20° head up tilt, with the head supported level with the trunk. The method of phrenic nerve pacing used is detailed in Chapter 5.

In Studies 2 & 5 (Chapters 4 & 7), after preparation of the skin by cleaning with alcohol, phrenic nerve activation was achieved with surface bipolar electrodes. The stimulators were applied behind the first head of the sternomastoid at approximately the junction of the lower and middle thirds of the muscle (Figure 2.3).

In Study 2 (Chapter 4), phrenic nerve stimulation was subsequently produced with percutaneous monopolar needle electrodes (Disa 13L61) by a method first described by Maclean and Mattioni (1981), and adapted by Aubier and colleagues (1985c).

FIGURE 2.3



A subject undergoing bilateral phrenic nerve stimulation with the bipolar electrodes (A) placed behind the first head of the sternomastoid, at the junction of the lower and middle thirds of the muscle. Also applied are electrodes for recording diaphragmatic electromyograms (B). A magnetometer (C) placed over the anterior rib cage is also visible. Although the abdomen is bound in this photograph, abdominal binding was not performed in the studies presented.

The needles were inserted through the skin at the site found on transcutaneous stimulation to give maximal phrenic nerve activation. The skin was infiltrated, prior to needle insertion, with 1% lidocaine to provide local anaesthesia. The indifferent electrodes were placed superficially, and the different electrodes were manoeuvred during stimulation to lie close to the phrenic nerve.

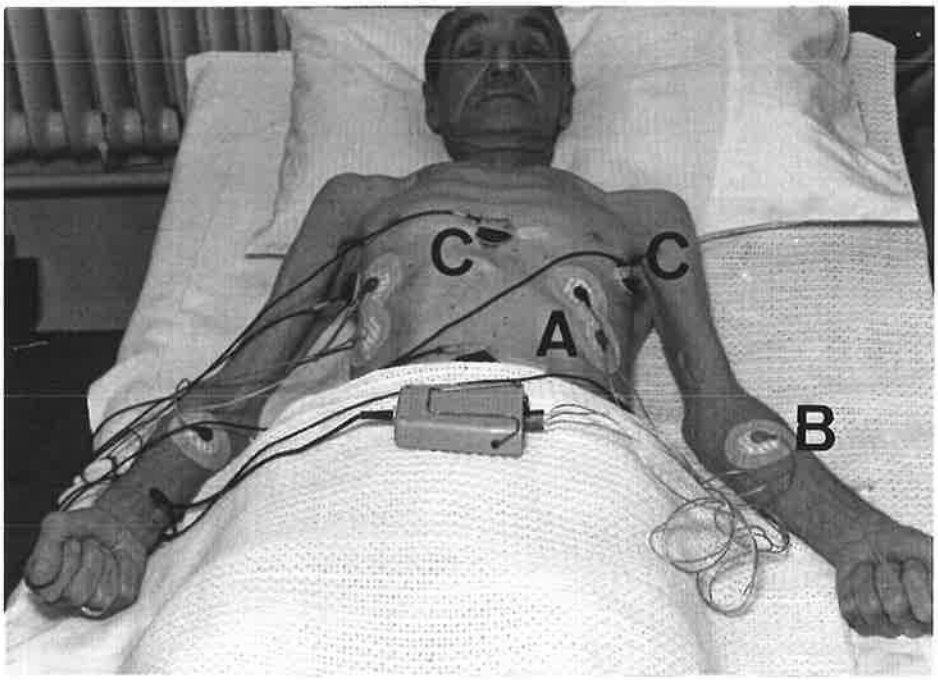
The orientation and site of the needle which produced greatest phrenic nerve activation was determined by carefully moving the needle during stimulation, and noting the height of the ipsilateral diaphragmatic electromyogram, after which the electrodes were secured firmly in place with tape. This procedure was performed separately for each side. In the same way the voltage to each side was increased to ensure maximal phrenic nerve activation, and remained above this optimal level, which was between 3 and 4 volts on each side, during measurement.

For both methods of phrenic nerve stimulation, the voltage producing maximal activation was checked two or three times during each study.

DIAPHRAGMATIC ELECTROMYOGRAM (EMG)

Electrical activation of the phrenic nerve was recorded as the diaphragm compound muscle action potential (diaphragmatic electromyogram [EMG]), with surface electrodes positioned in the right and left seventh and eighth intercostal spaces close to the costal margins and the mid-clavicular line (Figure 2.4).

FIGURE 2.4



A patient with superficial electrodes for the recording of diaphragmatic electromyograms (A) positioned in the left and right seventh and eighth intercostal spaces. A ground electrode (B) is placed on each arm. Rib cage magnetometers (C) can also be seen on the anterior and lateral chest walls.

Before recording, the skin was prepared by vigorous cleaning with an antiseptic skin preparation (Hibiscrub), and swabbing with alcohol.

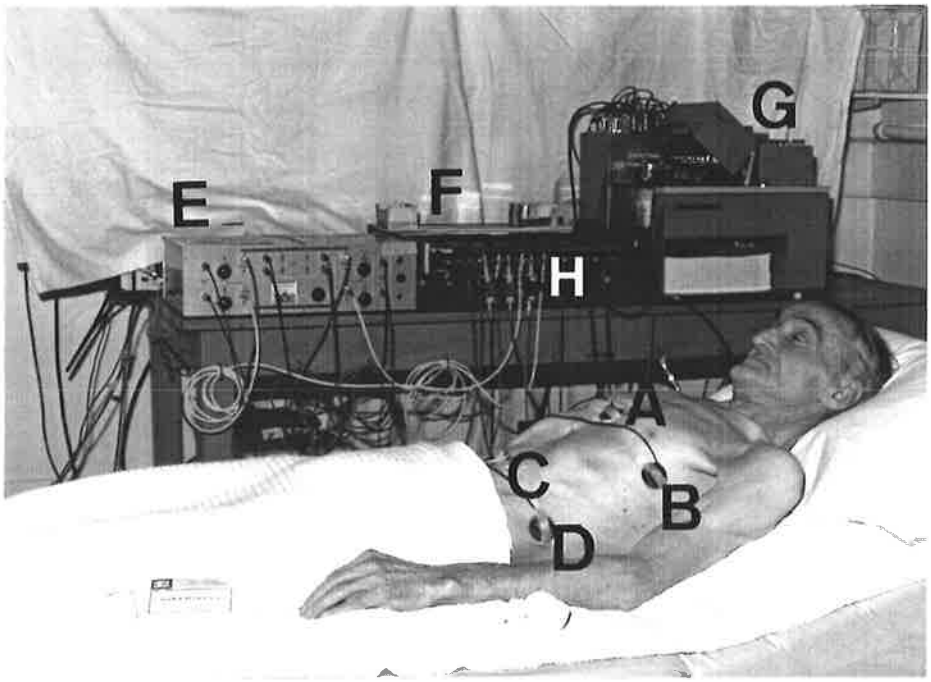
Having obtained the site of maximal activation from observation of the diaphragmatic electromyogram during gentle movement of the stimulating electrodes on each side, the voltage of stimulation was then increased until there was no further increase in either the electromyogram or twitch tension. The voltage was kept at least 10% above this optimal level during the subsequent study.

MAGNETOMETRY

Abdominal and thoracic wall movements were monitored during all respiratory manoeuvres with pairs of linearised magnetometers (Mead et al 1967).

Magnetometers were positioned at the level of the fifth intercostal space in the midline anteriorly and posteriorly to measure the rib cage anteroposterior diameter, and in the mid-axillary line 2-4 cms below the level of the anterior pair to measure the transverse diameter. The equivalent abdominal magnetometers were positioned 1cm above the umbilicus in the midline anteriorly and posteriorly, and at the same level on the right and left lateral abdominal wall (Figure 2.5). The magnetometers were attached to the skin with double-sided sticking discs (Medical Products 2181) and micropore tape. The coil axes for each pair of magnetometers were kept parallel throughout the investigations to minimise interference.

FIGURE 2.5



A photograph of a patient with COPD showing the position of anterior and lateral rib cage (A & B respectively) and abdominal anterior and lateral (C & D respectively) linearised magnetometers. The magnetometer box (E), magnetometer calibration ruler (F) (see Chapter 2, Section 2, Page 164), Mingograf pen and Racal tape recorders (G & H) can be seen in the background.

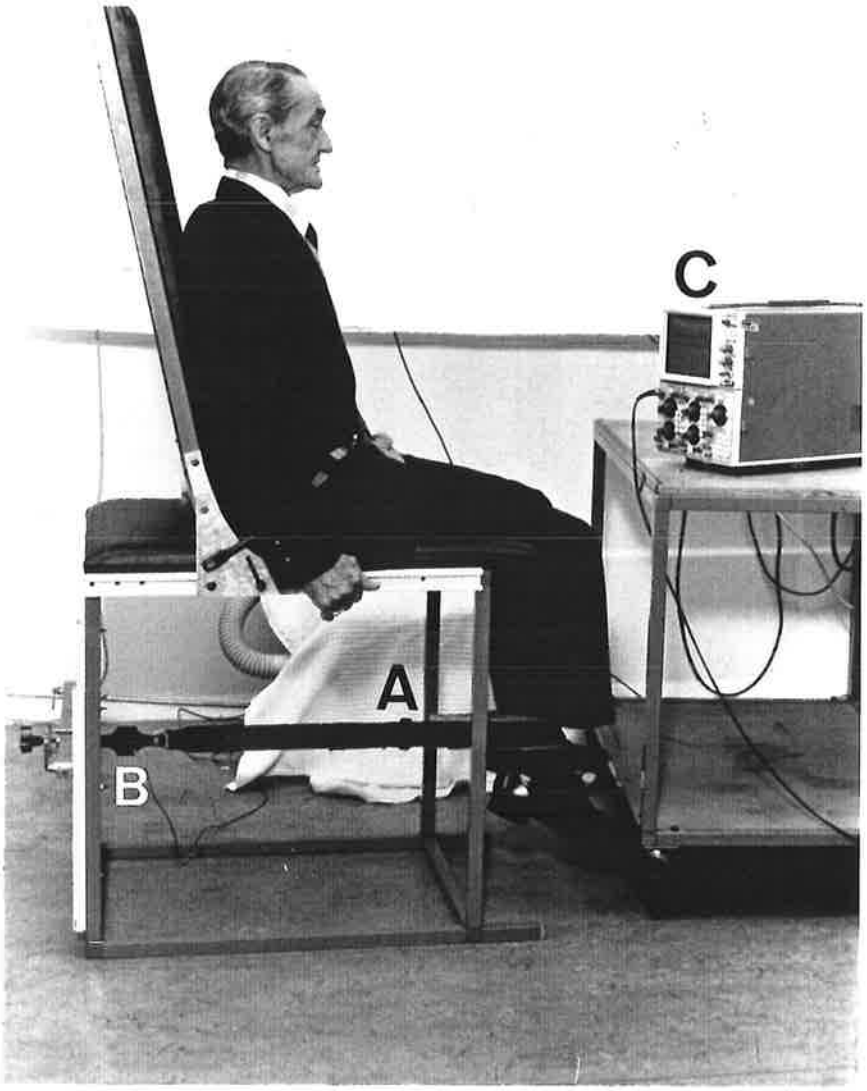
Following placement, the mean position of the range of movement for each pair of magnetometers was sited at end-expiration during tidal breathing for each body posture. This was checked and repositioned when the subject moved from seated to supine postures and vice versa during the study.

QUADRICEPS FEMORIS FORCE

The force of quadriceps femoris contraction was measured with subjects seated in a specially designed chair (Tornvall 1963). The back was adjusted so that the seat depth was the same as the length of the subject's thigh, and a belt around the hips secured the subject seated upright in the chair. A horizontal bar was positioned at the back of the chair to lie at the level of the subjects ankle when seated with the legs relaxed and the knee flexed to 90^{o}. A strain gauge was attached to the bar, and from it an inextensible strap passed around the ankle (Figure 2.6).

Subjects sat before the oscilloscope displaying the strain gauge output and, in the case of maximal voluntary contractions, were required to pull as forcefully as possible against the strap by straightening their leg at the knee (Edwards et al 1977a). The contraction was maintained for 2-4 seconds, and the greatest force sustained for one second was taken as the maximal contraction. The best three of 3-5 maximal voluntary contractions were recorded for each leg at intervals of 30 seconds between successive contractions.

FIGURE 2.6



A patient seated in the quadriceps femoris chair. The leg is at 90° to the thigh, and around the ankle is an inextensible strap (A) connected to the strain gauge (B) at the back of the chair. The patient faces the cathode ray oscilloscope (C) (Chapter 2, Section 2, Page 155) on which is displayed a trace of the quadriceps contraction.

The methods involved in the performance of quadriceps fatigue and endurance in Studies 1 and 4 respectively are detailed separately in Chapters 3 and 5.

PULMONARY FUNCTION MEASUREMENT

Pulmonary function measurements were performed to the normal routine of the Brompton Hospital, in the Respiratory Physiology Department under the management of Professor Denison.

They consisted of lung volume measurements including functional residual capacity, total lung capacity, residual volume, vital capacity, transfer factor and flow-volume loops from which forced vital capacity, forced expiratory volume in one second, and peak expiratory flow rate were recorded.

In Study 5, patients performed peak expiratory flow rate at each weekly visit. Two or three maximal efforts were performed with subjects seated, except in the case of one patient (No. 9), who preferred always to stand.

PLASMA THEOPHYLLINE LEVELS

Venous blood was taken from an antecubital vein at the end of each drug treatment period for each study, and for Studies 1, 4 & 5 (Chapters 3, 6 & 7), during the pretrial acclimatisation periods when the correct dosing schedules were being determined. Blood was taken between six and eight hours after oral active or placebo theophylline, and within 15-20 minutes after the end of the intravenous infusions for Studies 2 & 3 (Chapters 4 & 5).

Blood samples were spun down immediately and the serum frozen until assay in batches at the end of the studies. Analysis was performed in the Biochemistry Laboratories of the Brompton Hospital, using a fluorescence polarisation immunoassay with an Abbott TDX analyser and theophylline assay kits supplied by Abbott Laboratories.

MEASUREMENT OF STUDY TRACES

All pressure, spirometry and magnetometry traces were measured manually from the marked paper on which recordings were made, and using a decimal ruler. In the case of recordings known to follow a positive deflection, the ink trace was positioned with the top edge corresponding to the bottom border of the line on the recorder paper. For a negative deflection the bottom edge of the ink trace was positioned immediately at the top of the line on the recorder paper. In the case of maximal inspiratory and expiratory mouth pressures, a distance of one centimeter between the two outer edges of the ink-jet deflection was taken as the pressure maintained for one second. Uncommonly, ink blots obscured measurement, in which case the recording could be played off again and remeasured. On occasion, if the pressure was still difficult to measure, the recording was printed at a faster speed, thereby producing a narrow ink-jet trace.

SECTION 2 - EQUIPMENT

SPIROMETER

Lung volumes during respiratory muscle studies were recorded from a dry spirometer (Ohio 840) with a volume range of 0-10 litres.

CALIBRATION

Calibration was performed on test days with a three litre Vitalograph syringe in 500ml increments.

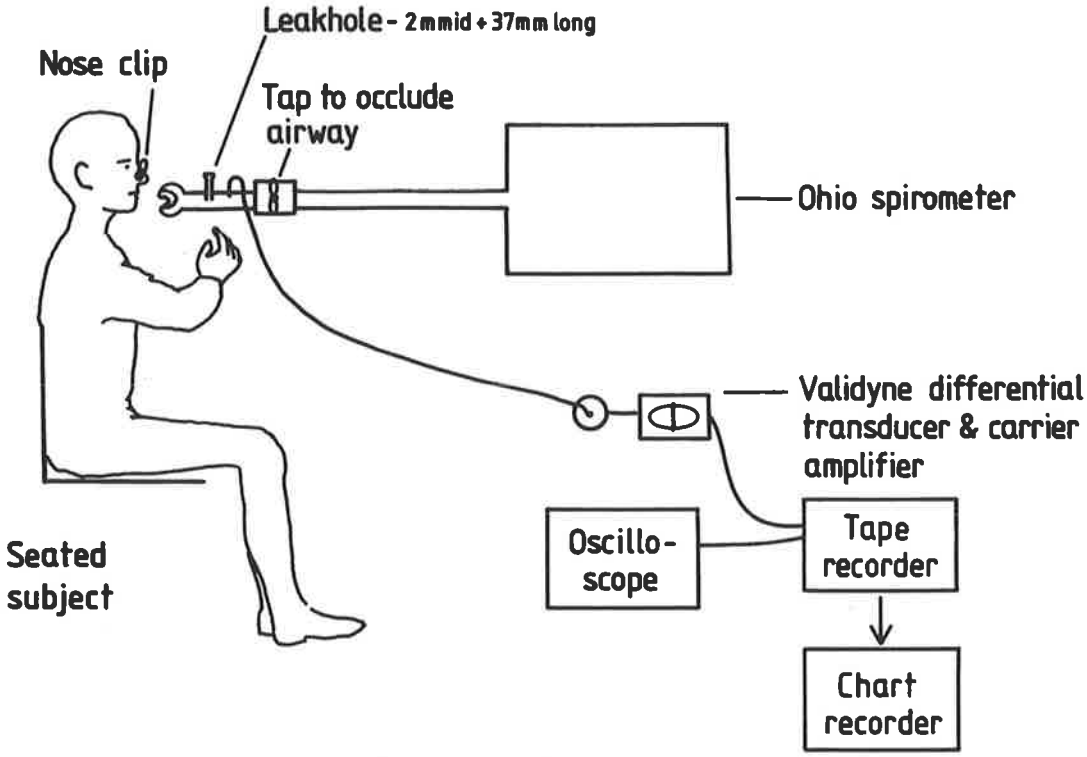
MAXIMAL STATIC MOUTH PRESSURE MEASUREMENT

The mouthpiece used for measurement of mouth pressures was adapted from a design by Black and Hyatt (1969). It comprised a copper tube three centimetres (cms) in diameter and 10cms in length.

A leak with dimensions of 2mm internal diameter and 37mm in length (Ringqvist 1966) was incorporated into the mouthpiece (Figure 2.7). This was to prevent glottic closure during performance of mouth pressure measurement, and so ensure that the values of the pressures measured were the same as those generated in the chest by the respiratory muscles, and were not influenced by the cheek muscles (Ringqvist 1966).

The mouth pressures were measured via an air-filled polyethylene catheter (90cms in length and 1.2mm internal diameter) with a differential pressure transducer (Validyne MP45-28, range ±500mmHg). The pressure signal was then amplified (Validyne CD12) prior to being recorded.

FIGURE 2.7



A diagram of the equipment used for measuring and recording maximal static mouth pressures. The small leak in the mouthpiece was utilised to prevent the development of pressure with the cheek muscles, and to prevent glottic closure. Prior to each maximal effort a vital capacity was performed. The subject then turned the tap to occlude the airway, and watched the effort on the oscilloscope. The pressure and volume traces were recorded simultaneously on tape and pen recorders.

CALIBRATION

Calibration was performed with a water-filled U-shaped manometer prior to each measurement session, by applying positive and negative pressures of magnitude 40cmsH_2O to the transducers.

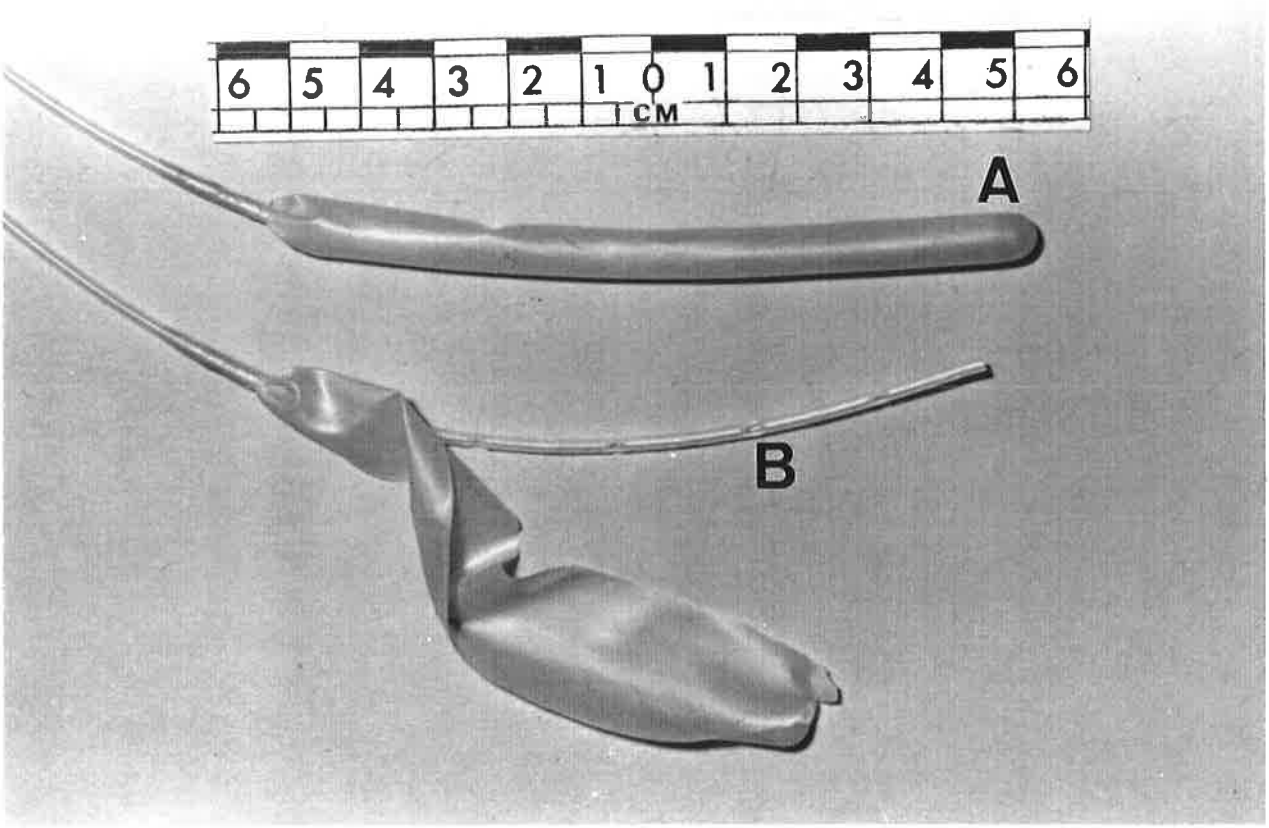
The transducer and amplifier had a linear response to $\pm$200cmsH_2O.

TRANSDIAPHRAGMATIC PRESSURE (PDI) MEASUREMENT

Pressures achieved in the oesophagus and stomach were measured with oesophageal and gastric balloon catheters. The air-filled catheters (No. 71510 P.K. Morgan) consist of flexible but firm tubing, 100cm in length and 1.2mm internal diameter with, at their proximal ends, a series of small perforations at intervals along the final 10cms of the tube. Encasing this end is a fine latex balloon, 10cm in length, a perimeter of 3.5cm and wall thickness of 0.06cm (Figure 2.8).

In Studies 3, 4 & 5, each catheter was attached to one side of a differential pressure transducer (Validyne MP45-1 range $\pm$ 150mmHg) and from there to Validyne amplifiers (Model CD12). In Study 2, the pressure transducer model was Validyne DP15. The signals were channeled through an electrical subtractor to give a value for transdiaphragmatic pressure.

FIGURE 2.8



The air-filled balloon catheters used for measurement of oesophageal and gastric pressures. The balloons consist of a latex sheath (A) surrounding the proximal end of the catheter. The lower catheter has had the balloon cut open to display the central polyethylene tubing in which are regularly spaced holes (B).

CALIBRATION

Calibration was performed with a water-filled U-shaped manometer prior to each measurement session, with pressures of ± 10, 20 and 50cmsH_2O (Appendix). In order to ensure that the electrical subtraction of oesophageal from gastric pressure gave correct values, varying pressures were applied simultaneously to both transducers (Appendix).

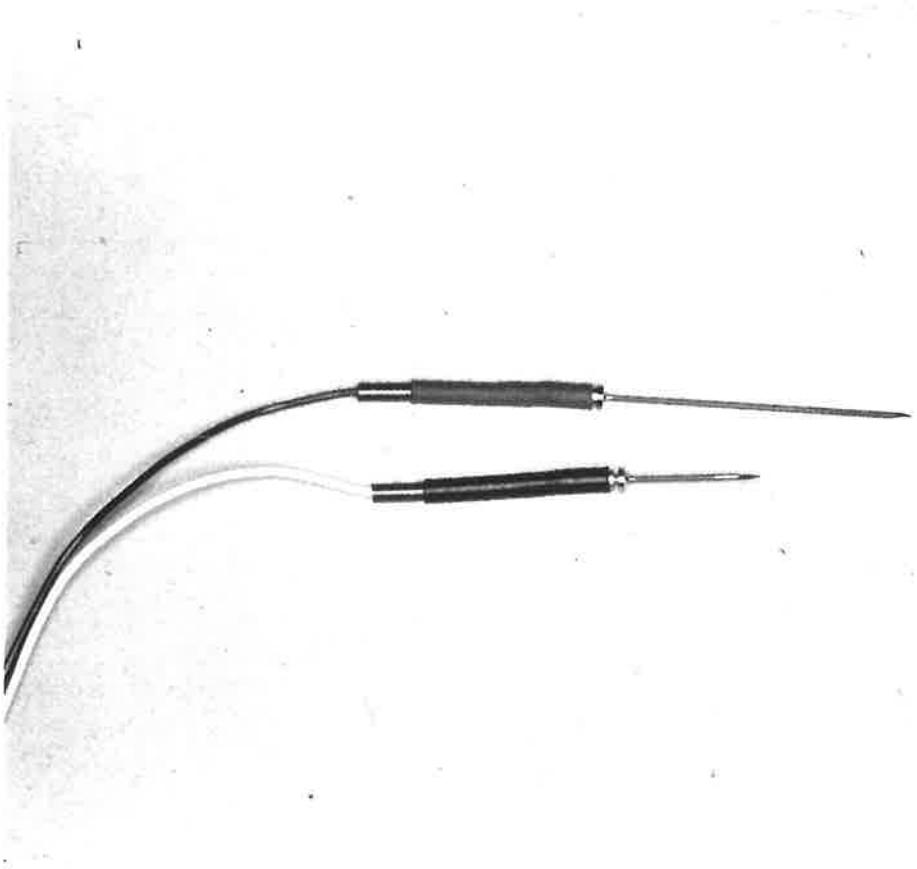
The pressure-volume characteristics of the balloon catheters were determined in water (Beardsmore et al 1980), which corresponds to the conditions in the oesophagus, and in air, corresponding to the milieu in the stomach. The ideal volume of air to be placed in the two balloons was 0.2-0.7 mls for the oesophageal balloon and 0.8-2.7 mls for the gastric balloon.

The catheters, transducers and amplifiers measured linearly over a range of ±200cmsH_2O.

PHRENIC NERVE STIMULATION

In Study 2, phrenic nerve stimulation was affected with unidirectional rectangular wave impulses of 1Hz at 3-4 volts delivered by two pairs of monopolar concentric stainless steel needles (Disa 13L61), teflon-coated with uncoated tips. The stimulating electrodes measured 35mm in length, and the indifferent electrodes were 15mm in length (Figure 2.9).

FIGURE 2.9



The Disa monopolar needle electrodes used for phrenic nerve stimulation in Study 2. The short electrode is the indifferent electrode, and the long needle the stimulating electrode. Both electrodes are insulated with teflon except at the tips. The wires connect to a gated stimulator.

In Studies 2 & 5, stimulation was achieved with unidirectional rectangular-wave impulses of 1Hz at voltages ranging from 60-160 volts. The impulses were produced by a dual output stimulator (Digitimer 3072), and applied percutaneously to the neck with two pairs of bipolar electrodes (Medelec 53054) with felt saline-soaked tips, 5mm in diameter and with an interelectrode distance of 12mm (Figure 2.10).

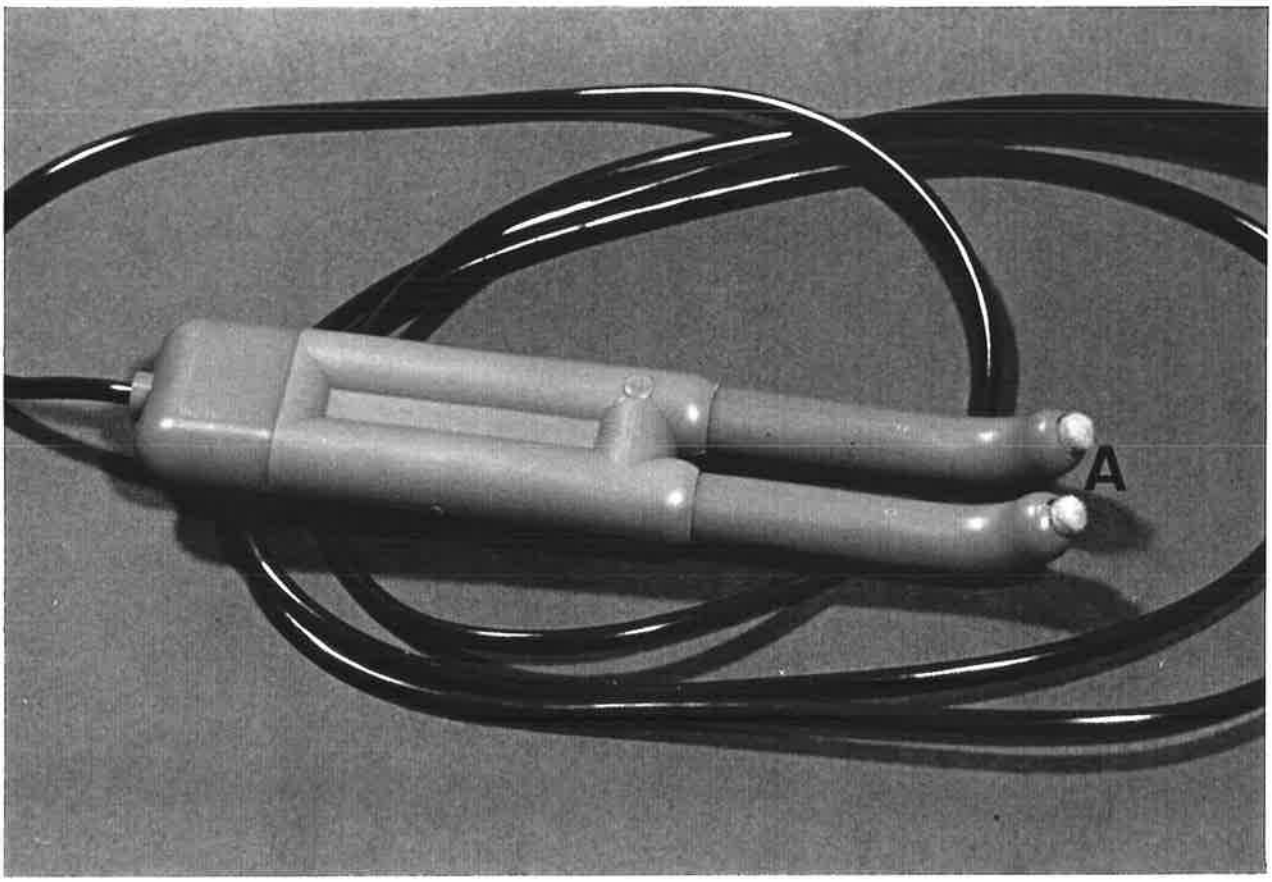
DIAPHRAGMATIC ELECTROMYOGRAM (EMG)

In Studies 3, 4 & 5, diaphragmatic electromyogram (EMG) was recorded via disc surface electrodes (Nikomed 460), and the EMG signal was passed through an amplifier (Medelec PA63) and filtered through a band of 16-1600Hz. In Study 2, the surface diaphragmatic electromyogram on each side were recorded via silver chloride disc electrodes placed in the sixth and seventh intercostal spaces, and the signals were passed through a Disa preamplifier (type 15C01) and then band-pass filtered (20Hz-2,000Hz).

CALIBRATION

Calibration was performed with an internal electrical signal at a variety of frequencies across the band-width of the amplifiers.

FIGURE 2.10



The Medelec stimulating bipolar electrodes used for phrenic nerve stimulation in Study 5, and prior to insertion of needle electrodes in Study 2. The felt pads (A) were soaked in saline prior to stimulation.

MAGNETOMETERS

The linearised magnetometers (Norman Petersen) (Chapter 2, Section 1, Page 141, Figure 2.5) consist of pairs of coiled wire in which electromagnetic fields are excited and received.

Interference between different pairs of magnetometers was minimised by using channels on the amplifier box with widely differing frequency bands for carrying signals from coils close to one another: 4.23 kilohertz and 5.91 kilohertz for rib cage anteroposterior and lateral diameters respectively, and 3.19 kilohertz and 4.82 kilohertz for abdominal anteroposterior and transverse dimensions respectively.

CALIBRATION

After each study the pairs of coils was calibrated over the distance corresponding to the diameters of the chest wall at functional residual capacity, taken as the zero position. The coils were attached to cardboard in a plane perpendicular to a ruler and parallel to one another. One coil was moved forwards and backwards, in 0.5cm increments from zero.

QUADRICEPS FEMORIS FORCE

The force transmitted to the strain gauge (Strainstall 1886D), range 0-100 kilograms (kg) (Chapter 2, Section 1, Page 143, Figure 2.6) was passed through an amplifier on the Mingograf 800 recorder prior to recording.

CALIBRATION

Calibration of the strain gauge was performed from 0-30kg with 10kg weights.

PULMONARY FUNCTION

Forced expiratory flow volume curves were recorded using a dry rolling seal spirometer (Ohio Spiroflow 131). Absolute lung volumes and airways resistance were measured in a whole body plethysmography (Fenyves & Gut). A 10 second single breath carbon monoxide transfer test was carried out with a PK Morgan model C apparatus. Normal reference values were from Cotes (1979), with the normal range taken as 85%-115% of the mean normal values.

RECORDING EQUIPMENT

All the measurements were performed with the subjects positioned in front of a storage cathode ray oscilloscope (Tektronix 5130N) (Chapter 2, Section 1, Page 143, Figure 2.6), on which was displayed traces of volume, pressure and magnetometer signals. There was a short delay between the effort and the display of the recording, so that patients in particular, might not limit effort if they believed a sufficient pressure had been achieved.

For all studies but Study 2, simultaneous recordings of lung volume, mouth pressure, transdiaphragmatic pressure and quadriceps force measurement were made on magnetic tape (Amplex) and a Racal Store 7 tape recorder with a frequency response of 5000Hz for later playback and analysis, as well as directly onto paper on an eight channel pen recorder (Mingograf 800, Siemens) (Figure 2.11).

In Study 2, all data were recorded directly onto a chart recorder (Gould Model ES 1000).

FREQUENCY RESPONSE OF BALLOON CATHETERS AND RECORDING EQUIPMENT

The frequency response of the entire measurement system of balloon catheters, transducers, amplifiers, electrical subtractor, Racal tape recorder and Mingograf chart recorder was tested, this being the system with the greatest number of pieces of equipment between the initial and final recording devices.

METHODS

The balloon catheters, filled with 0.5mls air, were placed inside an inflated party balloon which was sealed around the catheters. The measured pressure was above atmospheric pressure. The balloon catheters were connected to the transducers, which in turn were connected to the measuring equipment in the usual way. The party balloon was burst to create an immediate change in pressure, which had the effect of a square wave applied to the apparatus.

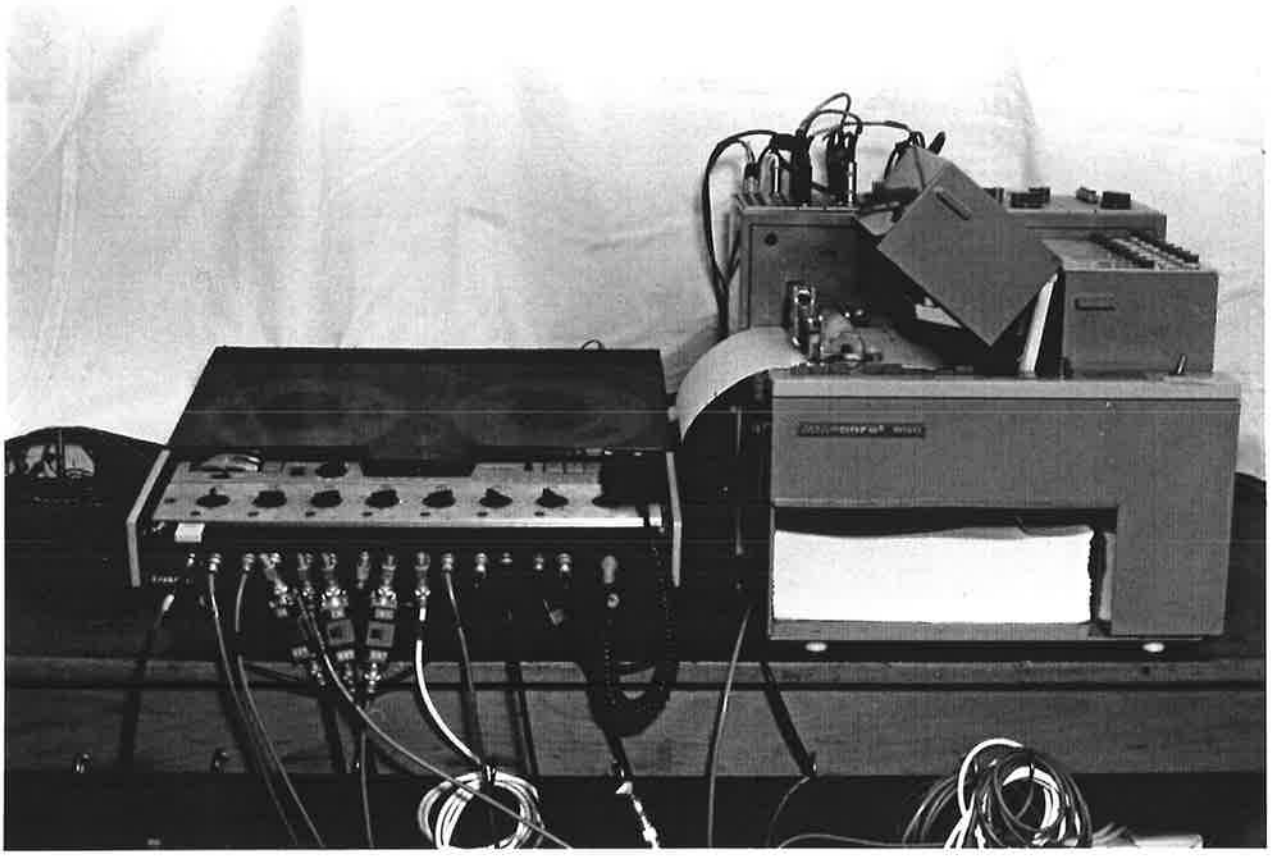
RESULTS

The 10-90% response time of the decline in pressure was used to calculate the frequency response which was 18.7Hz.

FREQUENCY RESPONSE OF MAGNETOMETERS

The channels used to record rib cage anteroposterior and transverse diameter and abdominal transverse diameter had a frequency response of 12Hz, while the channel used to record abdominal anteroposterior diameter had a frequency response of 32Hz.

FIGURE 2.11



The Racal tape recorder (left) and Mingograf pen recorder used for recording and playing-back data.

SECTION 3 - REPEATABILITY STUDIES: VALIDATION

MAXIMAL STATIC MOUTH PRESSURES

Prior to utilising respiratory mouth pressure measurements in studies, the repeatability of values taken over protracted periods was assessed in normal subjects, and in patients with lung disease. The objectives were:-

1. to determine the coefficients of variation (CV) of maximal inspiratory and expiratory mouth pressures in normal subjects and patients.

2. to ascertain whether a learning period was required by normal subjects and patients before the repeatability of between and within day measurements reached plateau levels.

3. to ensure that respiratory mouth pressure measurements were repeatable in the groups entered to studies of theophylline.

REPEATABILITY STUDIES - NORMAL SUBJECTS

SUBJECTS

The subjects were volunteers from the Cardiothoracic Institute, Brompton Hospital.

METHODS

Using the techniques described above in Chapter 2, Section 1, Pages 129-131, subjects performed 3-6 maximal expiratory mouth pressures (PEmax) and maximal inspiratory mouth pressures from residual volume (PImax) each day. The best three values for each manoeuvre were measured, and the single best value each day taken as maximum. Initially, mouth pressures were

performed on 2-3 days over a period of two weeks, then three times each week at regular intervals for the following fortnight, and then less frequently until measurements were recorded at intervals of as much as six weeks for a total duration varying between individuals from three to nine months. Thus, each subject performed PEmax and PImax measurements on at least 18 occasions.

STATISTICAL ANALYSIS

The three best values for PEmax and PImax on each day were meaned in order to determine within day repeatability, and the single greatest values on each day were used to determine between day repeatability. Previous studies of respiratory muscle strength have employed CV to gauge repeatability (Shephard et al 1958, Black & Hyatt 1969, Miller et al 1985); hence this method was used here to allow comparison of repeatability with these studies. The calculation was made by comparison of the standard deviation with its mean, and expressing the value as a percentage.

Coefficients of variation were calculated for each subject and for the group for the first three measurement days (Period A), the subsequent three study days (Period B), and another three days (Period C) following a break in measurement of several weeks. Period C included measurements made on three days separated by weekly intervals.

RESULTS

Six healthy volunteers (five females) aged 26-31 years were studied. All were familiar with respiratory function tests

but none were practised at the manoeuvres. Only two subjects had performed the tests on a previous occasion.

The mouth pressure measurements on all study days are given in Table 2.0. Coefficients of variation for within day and between day measurements were greater for Period A than for either Periods B or C (Tables 2.1 & 2.2). Mean within and between day CVs for PEmax were 9.9% and 12.5% for Period A, 6.4% and 5.0% for Period B, and 5.5% and 3.1% for Period C. The equivalent values for PImax were 9.0% and 12.0% for Period A, 5.1% and 7.0% for Period B, and 5.5% and 3.2% for Period C.

DISCUSSION

The subjects were not practised at the manoeuvres before entering the study. This is especially obvious from the PEmax results of Subject 4 during period A (Tables 2.0 & 2.1). Despite this, in all cases variability was satisfactory within a short time of first performing the mouth pressures.

PEmax and PImax correlate with age, weight, vital capacity effort and motivation and general fitness (Brody et al 1960, 1964, Leech et al 1983, Wilson et al 1984a), and as a consequence wide inter-individual variations in mouth pressures are expected. The variations found in this study are consistent with the wide normal ranges found by others (Shephard et al 1958, Craig 1960, Brody et al 1964, Ringqvist 1966, Black & Hyatt 1969), and make it difficult to draw conclusions from single values. Nevertheless, the repeatability of values is sufficient to allow measurements over time to be compared, and therefore the effect of an intervention to be assessed.

TABLE 2.0

PEMAX & PIMAX VALUES
($cmsH_2O$)

SUBJECT No.	DAY	PERIOD A	PERIOD B	PERIOD C
			PEMAX	
1	1	56, 60, 64	60, 64, 68	52, 58, 60
	2	46, 52, 54	60, 62, 66	50, 58, 60
	3	52, 60, 64	52, 54, 60	55, 60, 60
2	1	94, 100, 100	100, 106, 114	122, 126, 128
	2	132, 136, 136	108, 112, 114	116, 124 128
	3	120, 120, 122	102, 104, 114	120, 122, 128
3	1	68, 78, 82	98, 100, 104	94, 94, 96
	2	92, 96, 96	82, 106, 108	92, 94, 104
	3	60, 76, 84	76, 78, 88	82, 94, 100
4	1	38, 44, 68	62, 80, 92	68, 76, 88
	2	40, 50, 58	88, 90, 90	74, 80, 84
	3	62, 64, 66	80, 84, 88	72, 76, 76
5	1	80, 88, 90	108, 108, 116	100, 104, 110
	2	80, 88, 104	112, 116, 118	92, 100, 112
	3	100, 116, 126	120, 120, 128	104, 112, 112
6	1	64, 78, 78	80, 92, 104	98, 100, 104
	2	74, 78, 84	106, 110, 116	98, 104, 108
	3	90, 98, 106	106, 110, 112	108, 108, 108

TABLE 2.0 (cont'd)

SUBJECT No.	DAY	PERIOD		
		A	B	C
		PIMAX		
1	1	30, 36, 40	36, 40, 44	36, 36, 38
	2	32, 34, 36	34, 36, 40	32, 40, 40
	3	34, 36, 40	38, 42, 42	40, 40, 40
2	1	72, 76, 84	74, 74, 76	88, 94, 104
	2	100, 104, 108	80, 82, 84	80, 96, 102
	3	82, 82, 88	78, 82, 84	102, 106, 112
3	1	56, 56, 60	66, 66, 72	80, 80, 82
	2	50, 60, 72	64, 66, 72	70, 70, 76
	3	62, 68, 74	68, 70, 72	82, 84, 90
4	1	42, 42, 48	52, 58, 70	58, 64, 68
	2	46, 52, 52	56, 56, 60	62, 66, 70
	3	60, 66, 66	54, 56, 58	56, 60, 68
5	1	48, 56, 58	62, 62, 64	56, 60, 62
	2	50, 52, 56	48, 52, 52	60, 62, 64
	3	54, 60, 60	54, 56, 60	54, 56, 60
6	1	36, 36, 54	80, 82, 82	92, 96, 102
	2	54, 60, 64	88, 88, 96	100, 102, 102
	3	60, 62, 80	94, 98, 102	100, 102, 104

Maximal static expiratory and inspiratory mouth pressure values used to calculate coefficients of variation. The three best values on each study day were used, with between three and six values recorded at each measurement session. The three measurement periods, A, B and C, refer to the first three days of the study, the following three days, and three days several weeks subsequently.

TABLE 2.1

WITHIN DAY CV: PEMAX & PIMAX

SUBJECT No.	PEMAX PERIOD A	B	C	PIMAX PERIOD A	B	C
	COEFFICIENT VARIATION SD/MEAN (%)					
1	8.4	5.5	6.9	9.5	8.0	5.2
2	2.1	5.1	3.6	5.3	2.6	8.5
3*	9.5	8.6	5.8	10.3	4.2	3.7
4	20.7	8.5	7.5	6.7	8.1	8.0
5	10.4	3.6	6.3	7.2	3.9	4.6
6	8.5	6.8	2.6	14.9	3.5	2.8
MEAN	9.9	6.4	5.5	9.0	5.1	5.5

Within day coefficient of variation (CV) of the greatest three maximal static expiratory and inspiratory mouth pressures (PEmax & PImax) each day, expressed as a percentage (%). The three measurement periods, A, B and C, refer to the first three days of the study, the following three days, and three days several weeks subsequently. Coefficient of variation was calculated as the percentage (%) of the standard deviation to the mean value.

***Subject 3 was male.**

TABLE 2.2

BETWEEN DAY CV: PEMAX & PIMAX

SUBJECT No.	PEMAX PERIOD A	B	C	PIMAX PERIOD A	B	C
	COEFFICIENT VARIATION SD/MEAN (%)					
1	9.5	6.4	0.0	6.0	4.8	2.9
2	15.2	0.0	0.0	11.5	5.7	5.0
3*	8.7	10.6	8.2	11.0	0.0	5.2
4	8.3	2.2	7.4	17.1	10.3	1.7
5	17.0	5.3	1.0	3.4	10.4	3.2
6	16.5	5.5	2.2	23.2	11.0	1.1
MEAN	12.5	5.0	3.1	12.0	7.0	3.2

Between day coefficient of variation (CV) of the greatest daily maximal static expiratory and inspiratory mouth pressures (PEmax & PImax) on each of three days. The three measurement periods, A, B and C, refer to the first three days of the study, the following three days, and three days several weeks subsequently. Coefficient of variation was calculated as the percentage (%) of the standard deviation to the mean value.

***Subject 3 was male.**

REPEATABILITY OF MEASUREMENTS IN STUDY 4

METHODS

Those subjects entered to Study 4 (Chapter 6), were asked to perform PEmax and PImax as previously described, on at least three separate days before entering the study.

Within day and between day repeatability were calculated as described above for the first repeatability study.

RESULTS

Mean within and between day coefficients of variation for PEmax were 3.9% and 7.6% respectively, and for PImax were 4.4% and 7.4% respectively.

REPEATABILITY OF MEASUREMENTS IN PATIENTS

Patients with previously diagnosed interstitial lung disease admitted for routine assessment of their condition were studied. A group of patients with COPD and limited reversibility were assessed prior to their involvement in Study 5 (Chapter 7).

METHODS

The 24 patients with interstitial lung disease were studied on three separate days during their hospital admission, and of these, 10 were readmitted between one and five months later, and were studied on another three days during their stay.

The patients with COPD, who were all outpatients, came to the hospital at 2-21 day intervals for a minimum of seven visits. A series of 3-6 manoeuvres were performed each day for PEmax, and maximal inspiratory mouth pressures at residual

volume (PImaxRV) and functional residual capacity (PImaxFRC).

Within day and between day repeatability were calculated as previously described (Statistical Analysis, Page 159).

RESULTS

In the case of the patients with interstitial lung disease, there were 12 males and 12 females, age range 31-72 years, of whom 10 (three females, age range 35-66 years) were readmitted and restudied on a subsequent occasion. Aetiologies of the lung disease included cryptogenic fibrosing alveolitis (16 patients), sarcoidosis (6 patients) and scleroderma (2 patients). Lung volumes are presented in Table 2.3.

The group of 13 patients with COPD consisted of 10 males and three females aged between 42-73 years, the majority of whom (10, two females) subsequently entered Study 5. Pulmonary function for these patients is presented in Table 2.4.

Within and between day coefficients of variation for all patients with interstitial lung disease are presented in Tables 2.5. Within and between day coefficients of variation for PEmax were 9.6% and 14.2% respectively, for PImaxRV were 7.7% and 9.8% respectively, and for PImaxFRC were 9.1% and 12.6% respectively.

Table 2.6 gives results for the 10 patients studied on a second admission. The values on the first admission for this sub-group did not differ significantly from the group as a whole. The coefficients of variation showed a small improvement from the first to the second admission, which was generally at least three months later.

TABLE 2.3

PULMONARY FUNCTION – ILD

	MALES (n=12)	FEMALES (n=12)
	MEAN [SEM]	
TLC (Litres)	4.8 [0.3]	4.3 [0.3]
%Predicted	77.0 [4.7]	88.0 [4.2]
VC (Litres)	3.3 [0.3]	2.7 [0.2]
%Predicted	79.0 [5.7]	83.0 [4.6]
RV (Litres)	1.5 [0.1]	1.6 [0.1]
%Predicted	70.1 [5.6]	92.0 [5.8]

Pulmonary function of the patients with interstitial lung disease, expressed as absolute volumes in litres, and as percent (%) predicted. Total lung capacity (TLC), vital capacity (VC) and residual volume (RV) were measured. The patients had typical restrictive defects.

TABLE 2.4

PULMONARY FUNCTION - COPD

	MALES (n=10)	FEMALES (n=3)
	MEAN [SEM]	
TLC (Litres)	7.2 [0.5]	4.7 [0.1]
%Predicted	130.0 [8.0]	104.0 [1.1]
RV (Litres)	4.5 [0.4]	3.2 [0.1]
%Predicted	209.0 [13.6]	195.0 [2.5]
FRC (Litres)	5.7 [0.4]	3.8 [0.1]
FEV1/FVC (%)	34.3 [2.0]	39.3 [2.4]
% Reversibility	13.6 [3.3]	6.0 [2.6]

The pulmonary function of the 13 patients with chronic obstructive pulmonary disease (COPD) entered to the acclimatisation pre-trial period of Study 5, in order to assess the repeatability of mouth pressure and maximal voluntary quadriceps contractions. Total lung capacity (TLC), residual volume (RV), functional residual capacity (FRC), the ratio of forced expiratory volume in one second and forced vital capacity (FEV_1/FVC) were measured. The patients showed moderately severe obstructive defects.

TABLE 2.5

PEMAX & PIMAX CV – ILD

	MALES (n=12)	FEMALES (n=12)	ALL (n=24)
		(%)	
WITHIN DAY CV			
PEMAX	8.5	10.7	9.6
PIMAXRV	7.0	8.5	7.7
PIMAXFRC	8.7	7.7	9.1
BETWEEN DAY CV			
PEMAX	14.0	9.3	14.2
PIMAXRV	14.3	10.2	9.8
PIMAXFRC	11.2	14.1	12.6

Coefficients of variation (CV) of mouth pressure (Pm) measurements made on the same day (within day) and on different days (between day) on the first admission for 24 patients with interstitial lung disease. Maximal static expiratory mouth pressures (PEmax), maximal static inspiratory mouth pressures at residual volume (PImaxRV), and maximal static inspiratory mouth pressures at functional residual capacity (PImaxFRC) were performed.

TABLE 2.6

PEMAX & PIMAX CV – ILD

	MALES (n=6)	FEMALES (n=6)	ALL (n=10)
		(%)	
WITHIN DAY CV			
FIRST ADMISSION			
PEMAX	8.4	10.9	9.4
PIMAXRV	7.1	11.4	8.8
PIMAXFRC	10.1	13.0	11.3
SECOND ADMISSION			
PEMAX	7.6	7.6	7.6
PIMAXRV	6.8	11.1	8.5
PIMAXFRC	7.4	9.3	8.2
BETWEEN DAY CV			
FIRST ADMISSION			
PEMAX	15.9	15.2	15.6
PIMAXRV	10.3	10.6	10.4
PIMAXFRC	13.2	17.6	12.6
SECOND ADMISSION			
PEMAX	12.9	10.5	11.9
PIMAXRV	7.6	15.1	10.6
PIMAXFRC	11.4	16.4	13.4

Coefficients of variation (CV) on the first and second admissions for 10 patients with interstitial lung disease.

The results for the patients with COPD are presented in Tables 2.7. Within and between day coefficients of variation for PEmax were 7.6% and 7.3% respectively, for PImaxRV were 6.5% and 10.2% respectively, and for PImaxFRC were 6.1% and 10.7% respectively.

DISCUSSION

Repeatability for patients with interstitial lung disease was in keeping with that found in the studies of normal subjects (Tables 2.1, 2.2 & 2.5). For those patients studied on readmission the coefficients of variation were very similar, despite there being in some cases an interval of several months (Table 2.6).

All patients with COPD had severe airways obstruction and limited reversibility with 400 micrograms of salbutamol administered by metered dose inhaler (Table 2.4). The lower coefficients of variation for the patients with COPD compared to those for the patients with interstitial lung disease may be due to the relatively longer learning period used for the former patients.

The coefficients of variation were similar to those for normal subjects and allowed use of the parameters to assess treatment differences in subsequent studies.

TABLE 2.7

PEMAX & PIMAX CV - COPD

	MALES (n=10)	FEMALES (n=3)	ALL (n=12)
		(%)	
WITHIN DAY CV			
PEMAX	6.9	4.6	7.6
PIMAXRV	7.0	8.5	6.5
PIMAXFRC	6.4	7.5	6.1
BETWEEN DAY CV			
PEMAX	7.5	8.3	7.3
PIMAXRV	14.3	14.6	10.2
PIMAXFRC	10.3	9.1	10.7

Within day and between day coefficients of variation (CV) of maximal static expiratory mouth pressures (PEmax), maximal static inspiratory mouth pressures at residual volume (PImaxRV), and maximal static mouth pressures at functional residual capacity for patients with COPD.

MAXIMAL SNIFFS

The coefficient of variation in eight individuals for maximal measurements repeated on three days was 5.0% (Miller et al 1985).

PHRENIC NERVE STIMULATION

The reproducibility of unilateral and bilateral phrenic nerve stimulation has been assessed in our laboratory in four normal subjects lying supine (Mier et al 1985d, Mier 1992). Within day repeatability is 7.6% for unilateral twitches, and 7.5% for bilateral twitches; and between day repeatability is 10.1% for unilateral twitches, and 11.2% for bilateral twitches 11.2%.

QUADRICEPS FEMORIS MAXIMAL VOLUNTARY CONTRACTION (MVC) FORCE AND ENDURANCE

REPEATABILITY STUDIES

SUBJECT AND METHODS

In addition to the other measurements made on subjects and patients entered to Studies 4 and 5 (Chapters 6 & 7), left and right maximal voluntary quadriceps contractions (MVC) were performed on each day. At least three measurements were recorded and the best three values were used to calculate within day repeatability, while the single greatest value for each leg was used to determine between day repeatability.

For those subjects in Study 4, quadriceps endurance measurements were performed as described in Chapter 6, Methods, Page 253. These measurements were conducted after the MVCs,

with one leg studied in the morning and the other leg studied in the afternoon, the order being consistent throughout.

RESULTS

In the case of both subjects and patients, quadriceps MVC lay within the normal range of values (Edwards et al 1977a) (Table 2.8). The coefficients of variation were similar to those for the mouth pressure measurements (Tables 2.1, 2.2, 2.5, 2.7), and for other pulmonary function parameters. The coefficient of variation of quadriceps endurance measurements were substantially greater however, being an average of 27.3% for both legs.

DISCUSSION

The low coefficient of variation for quadriceps strength measurements in normal subjects and patients allowed use of the parameters to assess treatment differences in the subsequent studies. The much higher coefficients of variation for quadriceps endurance values made their reliability as an indicator of fatigue of the quadriceps inadequate, and consequently the recordings were not used in the study in patients.

TABLE 2.8

QUADRICEPS FEMORIS MVC & CV NORMALS & PATIENTS

	LEFT LEG	RIGHT LEG
NORMAL SUBJECTS	(n=5)	
WITHIN DAY CV (%)	3.5	4.2
BETWEEN DAY CV (%)	6.4	6.0
PATIENTS	(n=11)	
WITHIN DAY CV (%)	3.9	4.0
BETWEEN DAY CV (%)	5.8	6.7

Within day and between day coefficient of variation (CV) for maximal voluntary quadriceps contraction in normal subjects and in patients with COPD. Coefficient of variation was calculated as described previously as the percentage (%) of the standard deviation to the mean value.

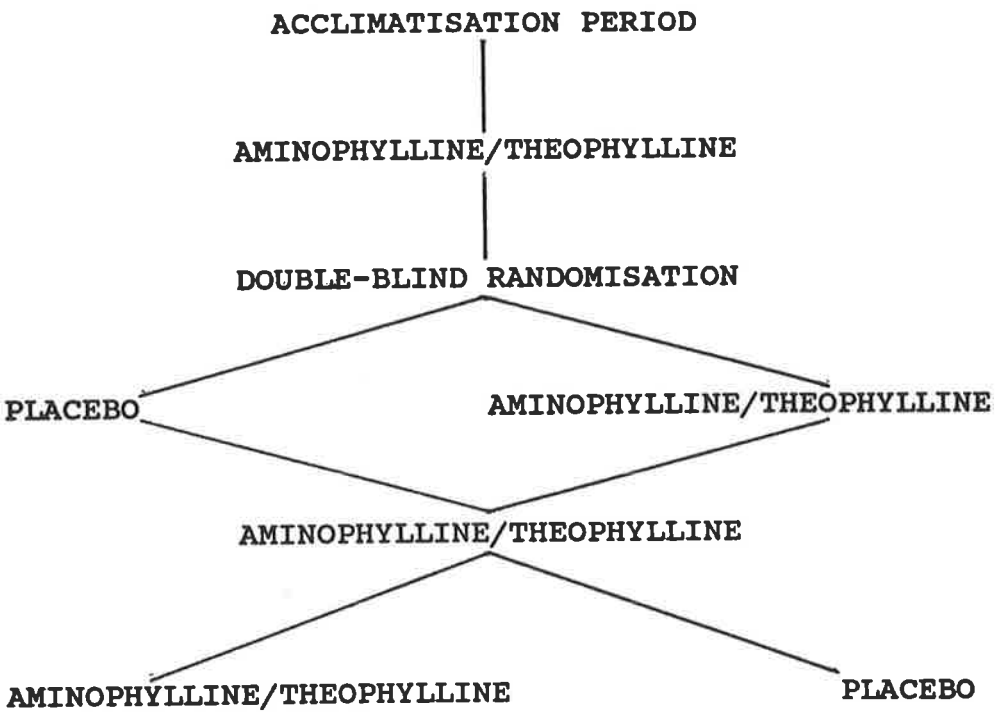
SECTION 4 - STUDY DESIGNS.

The studies of chronic dimethylxanthine administration conform to the same design: double-blind placebo controlled cross-over studies with each subject acting as his/her own control. This design was chosen because it produced controlled data and allowed comparisons to be made from small numbers of subjects. It was not possible to recruit large numbers of the highly motivated individuals required for the complex, effort-dependent and sometimes uncomfortable procedures.

An additional feature of these studies was the preliminary period during which subjects were dosed with the relevant dimethylxanthine before both the placebo and active treatment periods (Figure 2.12). This design has not been utilised in other published studies. It had the advantages of allowing the correct dose to be determined for each subject, and ensuring both that side-effects subsided before administration of the test medication and that the subjects were truly blind to the trial agent.

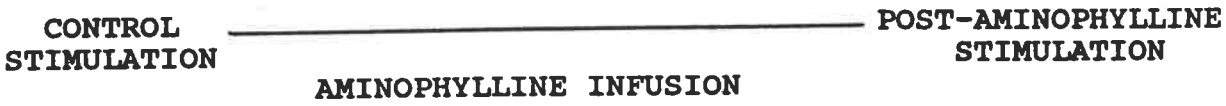
The two studies involving intravenous administration of aminophylline were open and uncontrolled, with measurements during a baseline period being compared to those following subsequent treatment (Figure 2.13). This design was considered appropriate because the cardiovascular and muscle effects of acutely administered aminophylline are easily identifiable by both subject and observer, rendering blinding of treatment impossible.

FIGURE 2.12



Protocol for the studies (Studies 1, 4 & 5) involving administration of oral aminophylline or theophylline over several days and weeks. The dosing with active drug before the two double-blind treatment periods ensured that the correct dose of dimethylxanthine was found for each subject, that tolerance to side-effects developed and that the subjects were genuinely blind to the subsequent treatment. During the acclimatisation period trial measurements were performed (except in Study 1), in order that any learning effect could occur before the treatment periods occurred.

FIGURE 2.13



The protocol for Studies 2 and 3 in which intravenous aminophylline was given acutely, in an open design. After a control period of phrenic nerve stimulation, the aminophylline infusion was administered, and a further period of phrenic nerve stimulation was performed.

CHAPTER 3

STUDY 1

THE EFFECT OF THEOPHYLLINE ON THE STRENGTH AND FREQUENCY-FORCE CURVE OF THE QUADRICEPS FEMORIS MUSCLE IN NORMAL SUBJECTS

INTRODUCTION

The skeletal muscle frequency-force relationship is utilised routinely *in vitro* as a means of determining the effect of an intervention. It is more difficult to generate frequency-force curves (FFCs) *in vivo* because they require the stimulation of a muscle either directly or through its motor nerve.

In the case of the diaphragm, the technique has been used by Moxham et al (1980) and Aubier et al (1981a); however neither study involved bilateral stimulation. The disadvantage of unilateral phrenic nerve stimulation is that the consequent asymmetric diaphragmatic contraction results in distortion of the chest-wall and the true interpretation of the effects of intervention may not be possible (Bellemare et al 1986).

Attempts in our laboratory to perform bilateral FFCs by stimulation of both hemidiaphragms simultaneously did not meet with success, because stimulation of the phrenic nerves in the neck produces powerful contractions of the sternomastoid muscles, which tend to lift the stimulating electrodes away from the phrenic nerves. Although it is possible to counteract this when performing unilateral stimulation, it is difficult to ensure that both electrodes remain in place during bilateral stimulation. In addition, the discomfort of bilateral stimulation make the studies unacceptable to the majority of subjects. Since these attempts were made, other workers have reported similar difficulties (Levy et al 1990).

Despite this, the frequency-force relationship provides an important means of investigating the direct effect of drugs on a muscle *in vivo*. A suitable skeletal muscle model was sought

which did not carry the disadvantages involved in studying the diaphragm. The quadriceps femoris was chosen because it is a large muscle, it is required to maintain postural tone for long periods, it has been studied before in man (Edwards et al 1977a), it is easily stimulated and its force is directly measurable.

AIMS

The primary aim was to determine the action of theophylline on the FFC of the quadriceps femoris, before and after the development of low frequency fatigue.

In addition, the effect of theophylline on the maximal voluntary contraction (MVC) of the quadriceps femoris before and after fatigue was assessed.

SUBJECTS

Healthy volunteers were selected from the staff and their associates of the Brompton Hospital.

METHODS

Subjects were seated in the quadriceps chair and MVCs were measured as noted in Chapter 2, Section 1, Pages 142 & 143 & Figure 2.6, Page 143.

Electrical stimulation of the quadriceps femoris was effected via 6" wide and 8" long (7" x 9" in the case of Subject 5) foil electrodes, wrapped in two-ply absorbent paper which was dampened with warm normal saline so as to keep the underlying skin and muscle close to blood temperature. The electrodes were placed over the anterolateral aspect of the

thigh (Edwards et al 1977a), and secured firmly with crepe bandages wrapped around the thigh, making sure that the bandages did not produce compression of the muscle and so interfere with blood supply.

Electrical leads passed from the stimulator to the electrodes and contact was achieved with crocodile clips. Stimulating square wave impulses of 50usec duration were produced with a Digitimer stimulator (type 3072). During the fatigue run (see below), a signal generator drove a gated pulse generator (Digitimer type 2521) which was connected to the stimulator, so ensuring that the stimulating impulses used were of the correct spacing, timing and duration. The rate of stimulation was monitored with a Universal counter (Racal 835) ensuring reproducible fatigue runs on each day. The frequency of stimulation during the production of FFCs was adjusted manually on the stimulator.

The contractile force and the stimulation frequency generated by the stimulator were recorded directly by the Mingograf pen recorder and via the Racal tape recorder on Ampex tape as previously detailed in Chapter 2, Section 2, Page 155 & Figure 2.11.

PROTOCOL

This was a double-blind placebo-controlled crossover study of chronic theophylline administration. The protocol followed was that described in Chapter 2, Section 4, Pages 174 & 175, Figure 2.12, except that an acclimatisation period was not applied to each subject. Instead, the production of FFCs was

tested in two subjects.

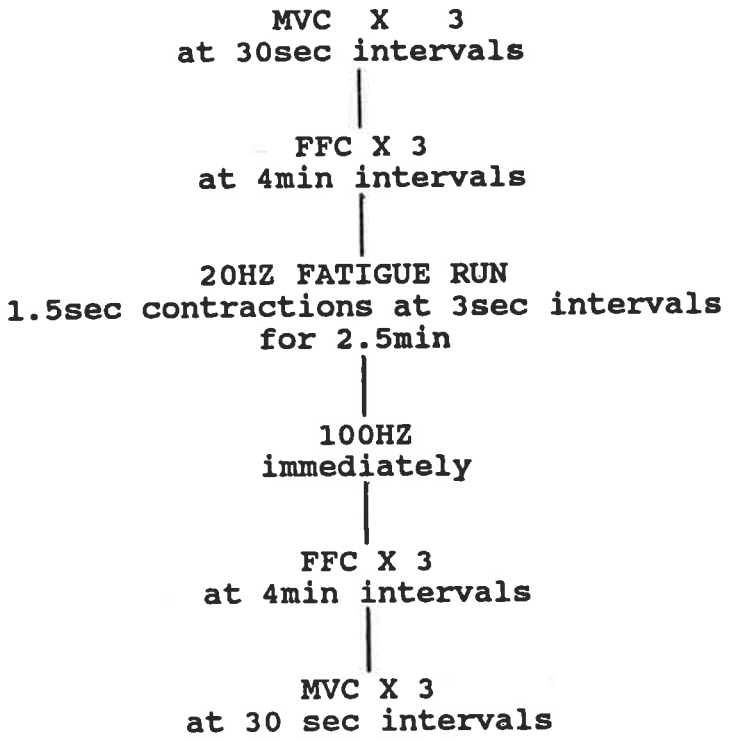
Oral theophylline (Uniphyllin, Napp Laboratories) was given for 3-5 days, after which time blood samples were taken for determination of serum theophylline levels. At the end of each 5-7 day treatment period the subjects attended the laboratory once for investigation.

Both legs were studied on each day, and the order of study was kept the same. The following measurement protocol was an adaptation of those by Edwards et al (1977a) and Wiles et al (1983). Each series of investigations consisted of at least three MVCs at 0.5-minute intervals followed, after two minutes rest, by three FFCs at four-minute intervals (Figure 3.1). The latter procedure was performed to ensure viable and repeatable FFCs were documented, and was successfully tested in two normal subjects prior to starting this study.

The FFC comprised trains of impulses lasting 1.5 seconds at 10, 20, 30, 50 and 100Hz. The voltage was that at which a 20Hz stimulation produced a contraction equal to approximately 30% of the greatest MVC for that day (Edwards et al 1977a).

Fatigue was then produced by stimulating the quadriceps at 20Hz intermittently for a period of 2.5 minutes with a train of impulses lasting 1.5 seconds each separated by a 1.5-second rest. Immediately following this fatigue run, a 100Hz stimulation was given, after which there was a rest period of one minute. Three FFCs followed and finally three MVCs were recorded, all performed in the same manner and at the same time intervals as before the fatigue run.

FIGURE 3.1



Measurement protocol for the production of maximal voluntary quadriceps contractions (MVCs), frequency-force curves (FFCs), before and after the production of low frequency fatigue by the 20Hz fatigue run.

Blood samples for theophylline assay were taken before the test procedures, approximately 12 hours after dosing.

MEASUREMENTS

The force developed during MVCs and all stimulated contractions was measured. In addition, the maximal relaxation rate (MRR), an index of the contractile state of the muscle (Wiles et al 1979a & b, Esau et al 1983a & b), was recorded for the stimulated contractions only.

STATISTICAL ANALYSIS

Comparison of the first, second and third FFCs before fatigue and the first, second and third after fatigue by paired t test, indicated an order effect, with statistically significant differences between the three sets of FFC. This was not recognized in preliminary work, when the technique and protocol were tested in two subjects prior to starting the study. The variability of the FFCs thus made it invalid to mean the data from the three FFCs before or after fatigue.

The different sets of FFCs each gave slightly different information about quadriceps muscle contractility. The first FFC before fatigue represented the fresh muscle, while the third gave information on the muscle immediately before the fatiguing run. After fatigue, the first FFC provided data on the muscle immediately after the fatigue run, and the third was performed when high frequency fatigue, which lasts for only a few minutes, should have subsided. There did not appear to be any specific value in analysing the second FFC, as it represented the contractility of the muscle for only a short

interval. It was feared that incorrect interpretations might arise from choosing one set of FFC over another set to compare the placebo and theophylline values. For these reasons, both the first and the third FFCs were individually analysed.

The mean force developed with MVC and for stimulated contractions of the first and third sets of FFC before and after fatigue and on both treatments were compared.

Maximum relaxation rate (MRR) may be altered in low frequency fatigue (Wiles et al 1979b). These workers analysed 30Hz contractions to determine MRR. In the current study, there was no difference in the values for the 20Hz and 30Hz contractions; hence for consistency, the values during 20Hz contractions are presented for the first and third sets of FFC performed before and after fatigue for both treatments, and for contractions at the start and end of the fatigue run.

The 20/100Hz ratio, which gives a numerical summary of the FFC (Edwards et al 1977a), and indicates the presence of low frequency fatigue (Efthimiou et al 1986), were also compared.

Mean data for the right and left legs were compared separately using paired t tests. Group data are presented as the mean and standard error of the mean (SEM). To compare differences for individual subjects two-way analysis of variance was performed for the right and left legs together.

RESULTS

Six healthy volunteers (three females) age range 30-39 (mean 33) years, entered the study.

MAXIMUM VOLUNTARY CONTRACTION (MVC)

Mean MVCs before and after fatigue and for the two test treatments are presented in Table 3.1.

The mean force after fatigue was statistically significantly less than that before fatigue for both legs on placebo, and for the left leg on theophylline ($P<0.02$).

Comparison of the measurements on the two treatment days indicate that the mean MVC before and after fatigue did not differ between placebo and theophylline.

FIRST FREQUENCY-FORCE CURVE

Difference between pre-fatigue and post-fatigue FFCs.

The first FFCs for each leg before and after fatigue, and for the two treatments are presented in Figure 3.2 (left leg) and Figure 3.3 (right leg).

The force at 100Hz before fatigue was approximately 38% of the pre-fatigue MVC. The force developed at 20Hz before fatigue was approximately 30% of the pre-fatigue MVC, and an average of 78% of the maximum stimulated force.

The production of low frequency fatigue can be identified from the change in the shape of the FFCs from before to after fatigue. Comparing the FFCs before fatigue with those after indicated statistically significant reductions in mean force at all frequencies of stimulation except at 10Hz for the right leg on placebo, at 50Hz for the left leg on theophylline, and at 100Hz for both legs on theophylline and on placebo.

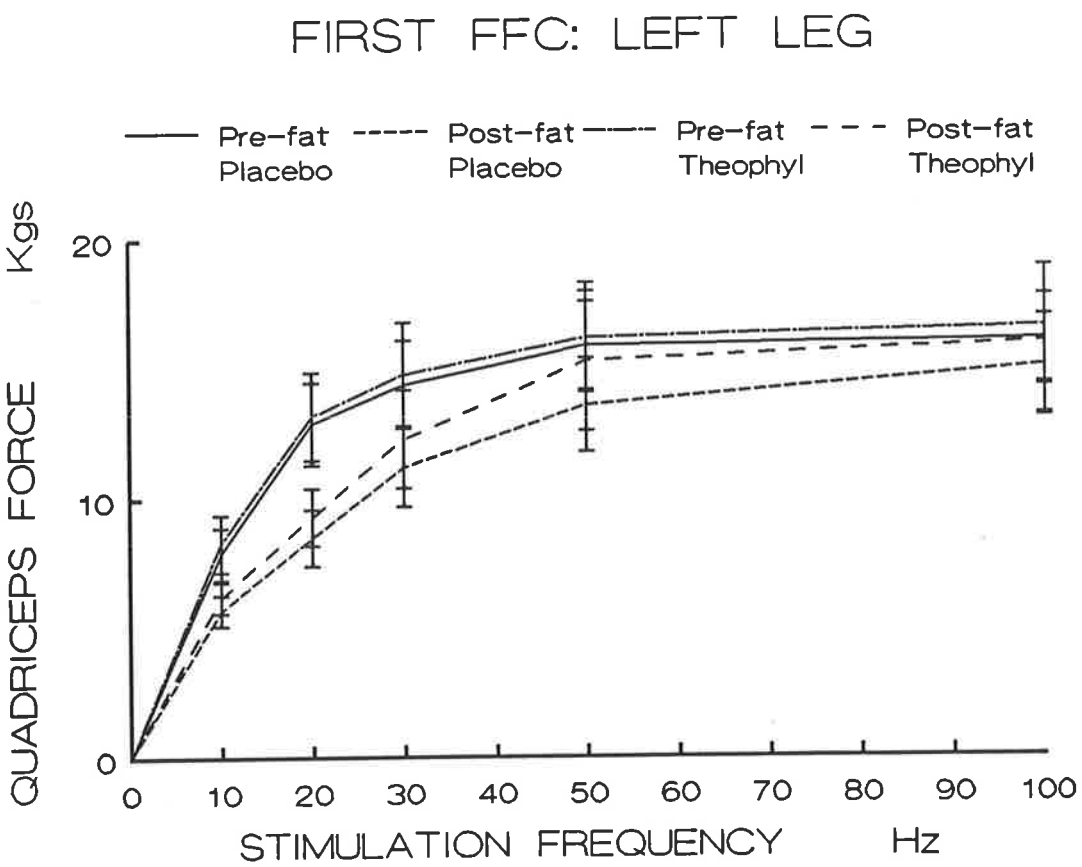
TABLE 3.1

MEAN MVC

	PLACEBO [SEM]	THEOPHYLLINE [SEM]
PRE-FATIGUE	(kgs)	
Lt leg	41.9 [5.1]#	44.7 [6.0]#
Rt leg	45.5 [5.7]#	45.0 [5.8]
POST-FATIGUE	(kgs)	
Lt leg	38.3 [5.8]	40.8 [6.3]
Rt leg	40.4 [5.4]	42.8 [6.5]

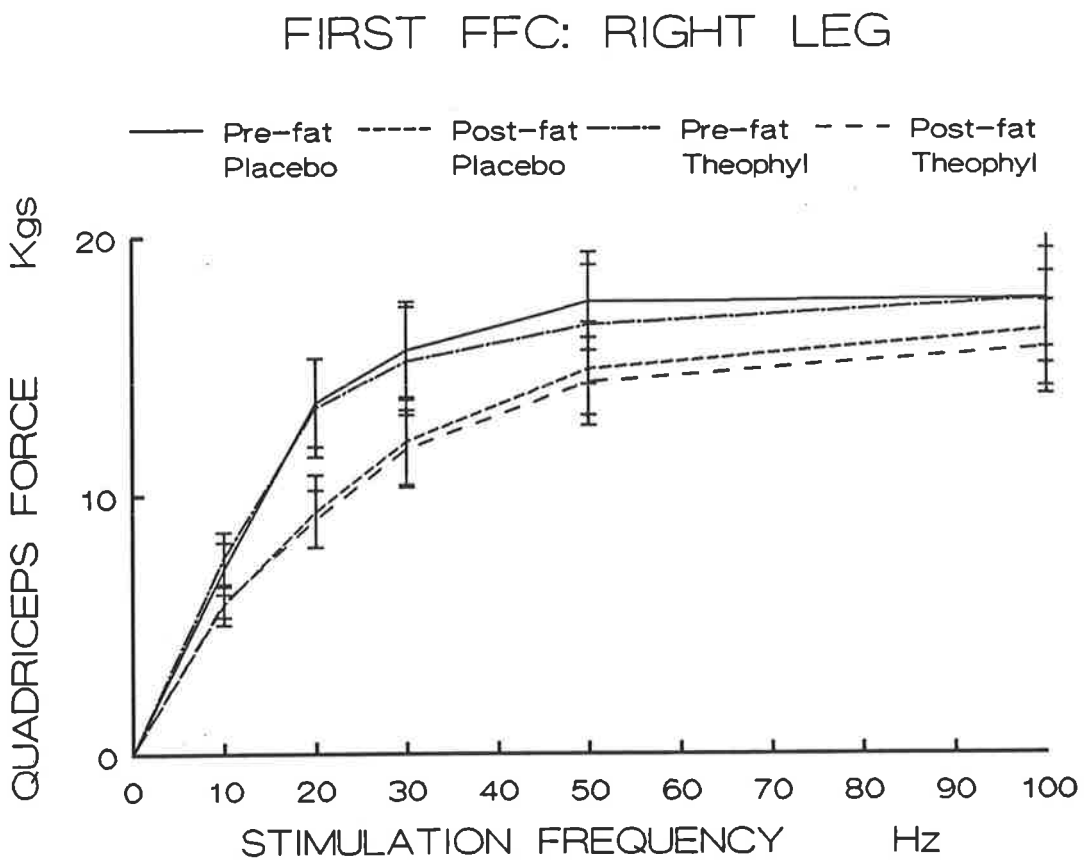
Mean maximal voluntary quadriceps contractions (MVCs) and standard error of the mean (SEM) for right and left legs before and after fatigue on the placebo and theophylline treatment days. Pre-fatigue MVCs for both legs on placebo and for the left leg on theophylline were statistically significantly greater than post-fatigue values (#P<0.02). There were no differences between placebo and theophylline treatment values.

FIGURE 3.2



The first force-frequency curves (FFCs) for the left leg before and after fatigue on placebo and theophylline, presented as the mean and standard error of the mean (SEM). There were statistically significant falls in force at low (<50Hz) frequencies of stimulation, after compared to before fatigue, on both treatment days (P<0.05). The difference between pre-fatigue and post-fatigue forces at 50Hz on placebo was also statistically significantly different. There were no treatment differences.

FIGURE 3.3



The first FFC for the right leg before and after fatigue, on both treatment days, as the mean and SEM. The pre-fatigue force at 10Hz on placebo did not differ statistically significantly from the equivalent post-fatigue force. Otherwise low frequency forces and force at 50Hz on both treatments showed a statistically significant fall from pre-fatigue to post-fatigue levels ($P<0.05$). There were no treatment differences for FFCs either before or after fatigue.

Moreover, the 20:100Hz ratios for both legs and on both treatments were statistically significantly less before compared with after fatigue ($P<0.005$; Mean both legs, before fatigue, 77.7%; after fatigue 53.1%. Table 3.2).

Comparing the mean MRR at 20Hz during the first FFCs, the values before fatigue were statistically significantly less than those after fatigue for the right leg only on placebo, and for both legs on theophylline ($P<0.02$) (Table 3.3).

Treatment differences.

There were no statistically significant differences between placebo and theophylline values in the force attained during the FFCs (Figures 3.2 & 3.3) or in MRR (Table 3.3). Similarly, the 20/100Hz ratios did not differ between treatment periods for the first sets of FFCs (Mean for both legs: on placebo, 77.0% before fatigue, 51.3% after fatigue; on theophylline, 78.3% before fatigue, 55% after fatigue. Table 3.2).

TABLE 3.2

MEAN 20:100Hz RATIO - FIRST FFC

	PLACEBO [SEM]	THEOPHYLLINE [SEM]
PRE-FATIGUE	% of pre-fatigue 100Hz value	
Lt leg	77.0 [5.0]#	80.1 [3.2]#
Rt leg	77.0 [1.7]#	76.5 [3.4]#
POST-FATIGUE	% of post-fatigue 100Hz value	
Lt leg	57.0 [3.4]	57.9 [3.4]
Rt leg	57.3 [1.5]	58.9 [3.7]

First FFCs: the ratios of force at low frequency compared to that at high frequency (20:100Hz ratio) expressed as a percentage for each FFC. The mean ratio and SEM before and after fatigue for the left and right legs on placebo and theophylline treatment days are presented. All pre-fatigue ratios were statistically significantly greater than post-fatigue values (#P<0.005). There were no treatment differences.

TABLE 3.3

MEAN MRR – FIRST FFC

	PLACEBO [SEM]	THEOPHYLLINE [SEM]
PRE-FATIGUE	% peak force loss/10msec	
Lt leg	11.2 [1.0]	11.8 [0.6]#
Rt leg	12.4 [0.6]#	12.4 [0.5]#
POST-FATIGUE	% peak force loss/10msec	
Lt leg	9.6 [0.6]	9.8 [0.6]
Rt leg	9.8 [0.3]	9.8 [0.6]

Mean maximum relaxation rate and SEM of 20Hz contractions during the first FFCs, for both legs and on both treatment days. Differences between pre-fatigue and post-fatigue values for the right leg on placebo and for both legs on theophylline were statistically significant (#P<0.02). There were no differences between placebo and theophylline values.

THIRD FREQUENCY-FORCE CURVE

Difference between pre-fatigue and post-fatigue FFCs.

The force at 100Hz before fatigue was approximately 38% of the pre-fatigue MVC. The force developed at 20Hz before fatigue was approximately 27% of the pre-fatigue MVC, and an average of 72% of the maximum stimulated force.

Most post-fatigue values were statistically significantly less than pre-fatigue values (Figure 3.4 & 3.5). The exceptions were for the right leg force at 50Hz and 100Hz on placebo, and for the right leg force at 100Hz on theophylline, the values for which did not differ (Figure 3.5). All differences between the pre- and post-fatigue FFCs at low frequencies (<50Hz) of stimulation were statistically significant.

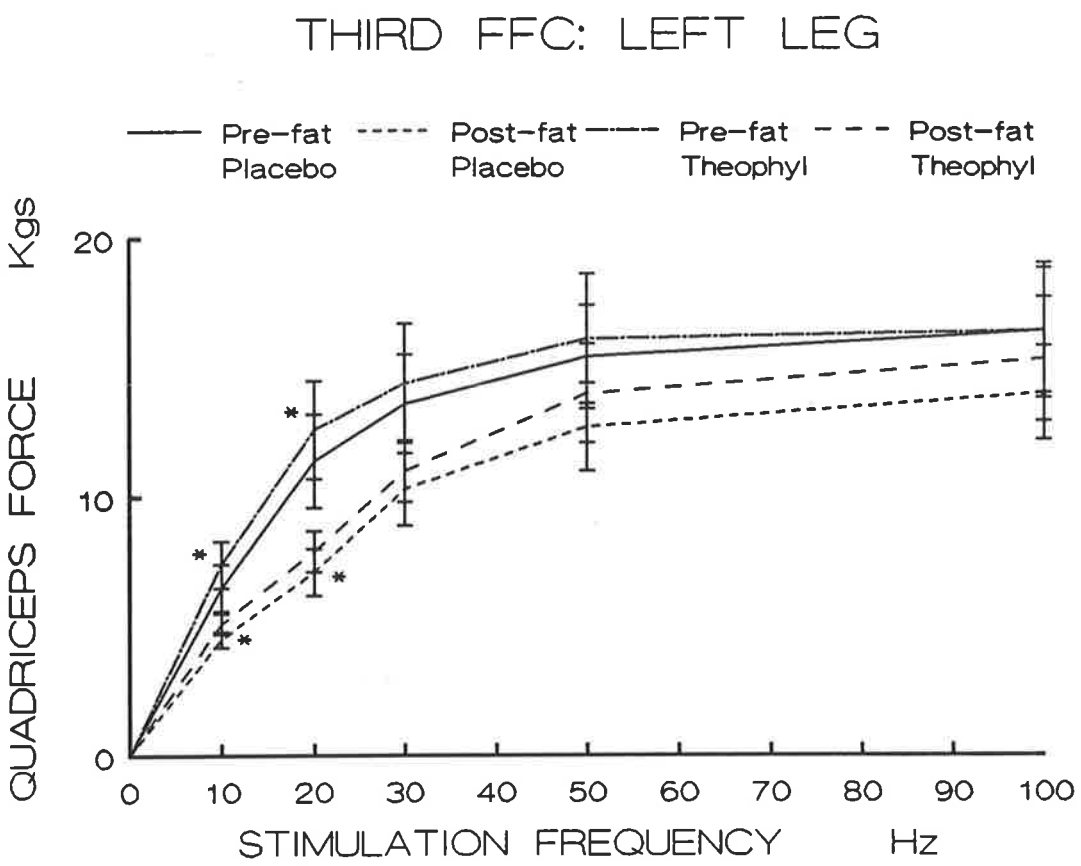
The 20:100Hz ratios for both legs fell statistically significantly from the pre-fatigue to the post-fatigue values ($P<0.005$, Mean for both legs before fatigue, 71.7%; after fatigue 46.9%. Table 3.4).

The difference between pre- and post-fatigue MRR was statistically significantly greater for the right leg on theophylline only ($P=0.021$) (Table 3.5).

Treatment differences

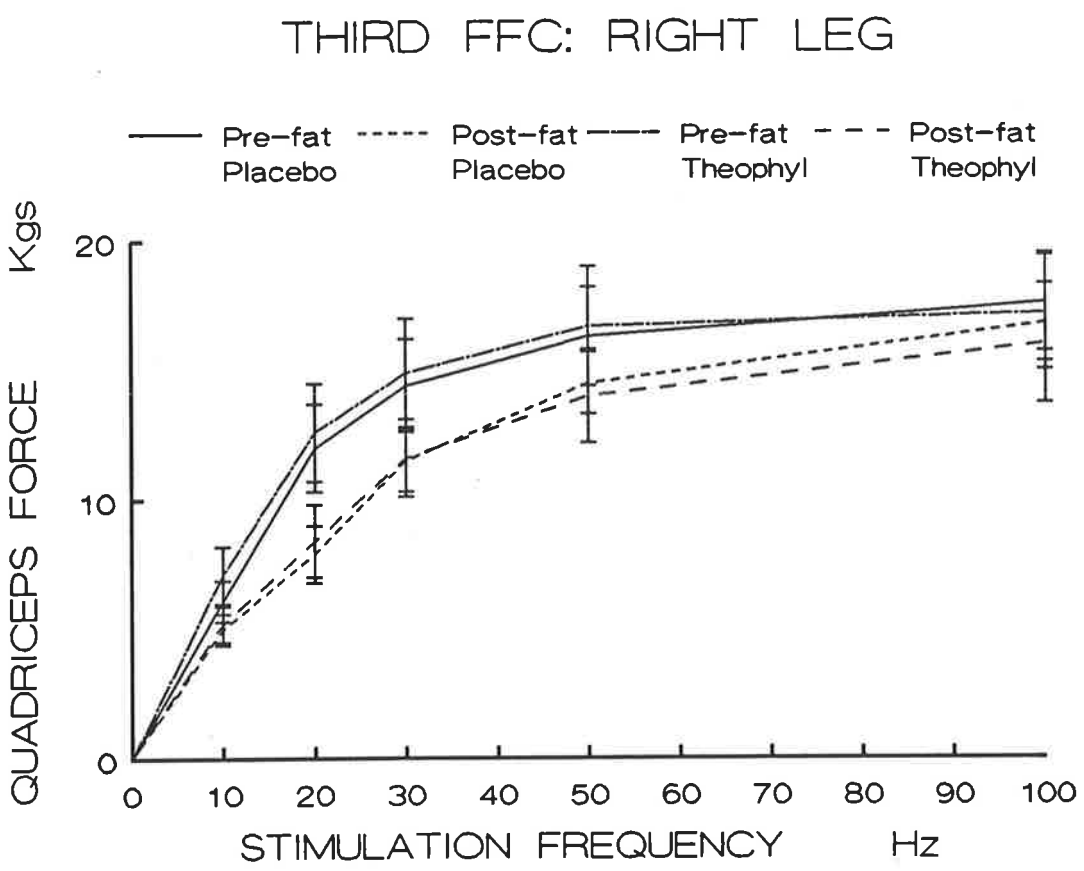
Comparing treatment days, there were statistically significant differences in the forces developed at 10Hz and 20Hz with the left leg before and after fatigue, with all values on theophylline being greater than the comparable placebo values (Figure 3.4).

FIGURE 3.4



The third FFCs for the left leg before and after fatigue and on placebo and theophylline, presented as the mean and SEM. All pre-fatigue values were statistically significantly greater than post-fatigue values. Theophylline values were statistically significantly greater than placebo values for 10Hz and 20Hz contractions pre-fatigue and post-fatigue (*P<0.05).

FIGURE 3.5



The third FFCs for the right leg before and after fatigue and on both treatments, presented as the mean and SEM. Pre-fatigue values were statistically significantly greater than post-fatigue values at all frequencies except at 50Hz and 100Hz on placebo, and at 100Hz on theophylline. There were no differences between placebo and theophylline values.

TABLE 3.4

MEAN 20:100Hz RATIO - THIRD FFC

	PLACEBO [SEM]	THEOPHYLLINE [SEM]
PRE-FATIGUE	% of pre-fatigue 100Hz value	
Lt leg	69.2 [3.2]#	77.5 [2.4]#*
Rt leg	67.4 [1.9]#	72.7 [1.9]#*
POST-FATIGUE	% of post-fatigue 100Hz value	
Lt leg	50.8 [1.6]	54.0 [3.6]
Rt leg	46.4 [2.6]	52.5 [2.8]*

Third FFCs: the ratios of force at low frequency compared to that at high frequency (20:100Hz ratio) expressed as a percentage for each FFC. The mean ratio and SEM before and after fatigue for the left and right legs on placebo and theophylline treatment days are presented. All pre-fatigue ratios were statistically significantly greater than post-fatigue values. The differences between theophylline and placebo ratios were statistically significant (*) for the left and right legs before fatigue (P=0.014, P=0.026 respectively) and for the right leg after fatigue (P=0.006).

TABLE 3.5

MEAN MRR - THIRD FFC

	PLACEBO [SEM]	THEOPHYLLINE [SEM]
PRE-FATIGUE	% peak force loss/10msec	
Lt leg	11.8 [0.6]	11.6 [0.7]
Rt leg	12.5 [0.6]	13.4 [0.8]#
POST-FATIGUE	% peak force loss/10msec	
Lt leg	11.1 [0.5]	10.0 [0.7]
Rt leg	11.2 [0.4]	11.2 [0.4]

Mean maximum relaxation rate and SEM of 20Hz contractions during the third FFCs, for both legs and on both treatment days. The difference between pre-fatigue and post-fatigue values for the right leg on theophylline was statistically significant (#P=0.021). There were no differences between placebo and theophylline values.

The pre-fatigue 20/100Hz ratios were statistically significantly greater on theophylline compared to placebo for both left and right legs (P=0.014, left leg, 69.2% on placebo; 77.5% on theophylline; P=0.026, right leg, 67.4% on placebo, 72.7% on theophylline). The same was true for the post-fatigue ratio for the right leg (P=0.025, 44.5% on placebo; 48.3% on theophylline) (Table 3.4).

There were no treatment differences in maximal relaxation rate either before or after fatigue (Table 3.5).

FATIGUE RUN

Difference between pre-fatigue and post-fatigue values.

The production of low frequency fatigue by the fatigue runs was evident from the statistically significant reduction in mean force at 20Hz (P<0.005, mean both legs, before fatigue, 11.7kgs on placebo, 12.6kgs on theophylline; after fatigue, 7.4kgs on placebo, 7.7kgs on theophylline) (Table 3.6), and in mean MRR at 20Hz (P<0.005, mean both legs before fatigue, 11.5% plateau loss/10msecs; after fatigue, 5.9% plateau loss/10msecs) (Table 3.7) from the beginning to the end of the runs.

Treatment differences.

Left leg force at the start of the fatigue run was statistically significantly greater on theophylline than on placebo (P=0.011, on placebo, 10.9kgs; on theophylline, 13.0kgs) (Table 3.6). Maximum relaxation rates on the two treatments did not differ either at the start or at the end of the fatigue run (Table 3.7). The rate of decline in force during the fatigue runs did not differ between treatments.

TABLE 3.6

MEAN FORCE - FATIGUE RUN

	PLACEBO [SEM]	THEOPHYLLINE [SEM]
START FATIGUE RUN	(kgs)	
Lt leg	10.9 [1.7]#	13.0 [1.9]#*
Rt leg	12.5 [1.4]#	12.2 [1.8]#
END FATIGUE RUN	(kgs)	
Lt leg	6.7 [0.7]	7.7 [0.9]
Rt leg	8.0 [1.0]	7.7 [0.6]

The mean force and SEM during the first and last 20Hz contractions of the fatigue run for the left and right legs. All values at the start of the fatigue run were statistically significantly greater than those at the end (#P<0.005), indicating that fatigue occurred. The differences between placebo and theophylline values were not statistically significant except for the left leg at the start of the fatigue run (*P<0.011).

TABLE 3.7

MEAN MRR - FATIGUE RUN

	PLACEBO [SEM]	THEOPHYLLINE [SEM]
START FATIGUE RUN	% peak force loss/10msec	
Lt leg	11.4 [0.9]#	10.7 [0.4]#
Rt leg	11.6 [0.4]#	12.4 [0.7]#
END FATIGUE RUN	% peak force loss/10msec	
Lt leg	5.7 [0.5]	5.5 [0.4]
Rt leg	6.0 [0.2]	6.3 [0.4]

Mean maximal relaxation rate and SEM for the first and last 20Hz contractions of the fatigue run for the left and right legs on placebo and theophylline. All values at the start of the run were statistically significantly greater than those at the end (#P<0.005). There were no treatment differences.

INDIVIDUAL SUBJECTS

All subjects showed statistically significant decreases in the 20/100Hz ratios after fatigue compared with before, on both treatment days. In addition there was preservation of force at the highest frequencies of stimulation (50Hz and 100Hz) during both the first and third FFCs for all subjects.

None of the subjects had statistically significant differences in the 20/100Hz ratios of the first FFC between placebo and theophylline. However, in the case of the third sets of FFCs for both legs together, four subjects (Subjects 1, 3, 4 and 5) had statistically significantly greater values on theophylline than on placebo.

COMPARISON OF FIRST AND THIRD FREQUENCY-FORCE CURVES

Each Frequency

Comparison of mean force at each frequency revealed statistically significant differences between the pre-fatigue force at 10Hz and 20Hz for both legs on placebo, at 10Hz for the left leg and 20Hz for the right leg on theophylline, with greater forces being recorded for the first FFC. With higher frequencies no statistically significant differences attributable to the order of FFC were seen.

After the fatigue runs, similar differences were seen: at 10Hz and 20Hz for the left leg on placebo and on theophylline, and at 20Hz for the right leg on placebo. In all cases the forces during the first FFCs were higher than those during the third FFCs. No such changes were seen in MRR.

Individual Subjects

When the first and third FFCs for each individual were compared, there were statistically significant differences for subjects 1, 3 and 6 before fatigue, and for subjects 2, 3 and 5 after fatigue, with the first FFCs at both time points giving greater forces than the third.

Treatment differences

The mean size of treatment difference between the fall in force at 20Hz from the first FFC before the fatigue run to the third FFC before the fatigue run was 2.2% (expressed as a percentage of the pre-fatigue MVC on each day).

After the fatigue run the mean difference between theophylline and placebo days for the fall in 20Hz contractile force from the first to the third FFC was 0.75%.

Before and after fatigue the differences favoured theophylline.

THEOPHYLLINE LEVELS

All but one subject (No. 3, 9.8mg/l) had blood levels within the therapeutic range. The mean and standard deviation was 13.7mg/l ±3.5, with a range of 9.8-18.9mg/l.

No side effects were reported.

DISCUSSION

LOW FREQUENCY FATIGUE

The comparison of mean pre- and post-fatigue forces indicate that fatigue was produced by the regimen of intermittent stimulation at 20Hz.

The evidence for the production of low frequency fatigue in this study is:

1. the reduction in forces at low frequencies (<50Hz) of stimulation after fatigue, with no differences at high frequencies. This is illustrated by the shift in the frequency-force relationship down and to the right (Figures 3.2-3.5).

2. the reduction in 20:100Hz ratio after fatigue (Tables 3.2 & 3.3).

The greater reduction in force at low than at high frequencies of stimulation is a cardinal feature of low frequency fatigue.

Comparison of pre- and post-fatigue mean forces at 10Hz for the right leg during the first FFC showed that the direction and size of change was similar to that seen at other low frequency forces during both the first and third sets of FFC. Although the difference was not statistically significant ($p=0.057$), it was in keeping with the development of low frequency fatigue.

3. the reduction in 20Hz force from the start to the end of the fatigue run (Table 3.6).

4. the fall in maximum relaxation rate from the beginning to the end of the fatigue runs (Table 3.7).

Maximal relaxation rate has been used as an indicator of fatigue. However, it appears that the fall with fatigue persists for only 1-5 minutes (Esau et al 1983a). During the fatigue run the maximum relaxation rate gradually declined, and in all individuals on both study days there were significant differences between the values at the beginning and end of the

fatigue run. By the time the first FFCs after fatigue were produced, the maximum relaxation rate had returned to pre-fatigue levels in some individuals. During the third FFC post-fatigue, mean maximum relaxation rate did not differ from pre-fatigue levels. In keeping with the work by Esau and others (1983a), a short rest was sufficient to restore the rate to normal levels.

There was a fall in mean maximum voluntary contraction after fatigue. This was likely to be due to central fatigue rather than peripheral fatigue, as the 100Hz force levels during the FFCs generally remained normal after fatigue.

TREATMENT EFFECTS

Theophylline had no effect on fresh muscle. The evidence for this is:

1. there were no differences between pre-fatigue MVCs on the placebo and theophylline study days (Table 3.1).

2. there were no treatment differences in the percentages of MVC achieved with 20Hz and 100Hz stimulation before fatigue (Figures 3.2-3.5).

3. there was no difference between forces or the 20/100Hz ratios of the first pre-fatigue FFCs on the two treatments (Figures 3.2 & 3.3, Table 3.2).

Theophylline did not prevent fatigue occurring because:

1. there were no treatment differences between the values of the 20Hz contraction for both legs at the end of the fatigue run. This was despite a greater value for the left leg 20Hz contraction force on theophylline at the start of the fatigue

run (Table 3.6).

2. there were no treatment differences between the maximum relaxation rates for both legs at the beginning and the end of the fatigue runs on the two treatments (Table 3.7).

3. the lack of differences in the 20:100Hz ratios of the first post-fatigue FFCs (Table 3.2).

Theophylline did produce a slight attenuation in the extent of fatigue evidenced from:

1. the greater forces on theophylline at 10Hz and 20Hz during the third pre-fatigue FFC for the left leg (Figure 3.4).

2. the occurrence of differences between the first and third pre-fatigue FFCs occurred at 20Hz for the left leg and at 10Hz for the right leg on placebo but not on theophylline.

Pre-fatigue force levels at 10Hz and 20Hz during the first FFC were greater than the corresponding forces during the third pre-fatigue FFC. These differences in the two FFCs indicate that, to a small extent, fatigue was produced by the performance of the FFCs themselves. On placebo, differences between the two FFCs were seen for both legs at both stimulation frequencies. On theophylline equivalent differences were seen for the left leg at 10Hz and for the right leg at 20Hz only.

3. the treatment difference in forces at 10Hz and 20Hz during the third post-fatigue FFC for the left leg (Figure 3.4). No such differences were seen for the first FFCs on the two treatment days however, suggesting that the effect was

small (Figure 3.2).

4. the greater 20:100Hz ratio on theophylline for the right leg during the third post-fatigue FFC (Table 3.4).

5. the lack of difference between the forces at 20Hz during the first and third post-fatigue FFCs for the right leg on theophylline.

Such a change was seen on placebo for both legs and for the left leg on theophylline. The phenomenon suggests that further fatigue was produced by the performance of the FFCs themselves, as appeared to be the case for the pre-fatigue FFCs, or merely that low frequency fatigue continued to develop after the completion of the fatigue run. In the right leg, theophylline appeared to halt the development of further fatigue.

Overall, the size of the treatment effect was small with a benefit in terms of the force developed at 20Hz relative to the prefatigue MVC of 2.2% before fatigue and 0.75% after fatigue.

These differences indicate a relative increase in the amount of force developed with theophylline at low frequencies of stimulation. It is low frequency fatigue that is thought to occur in patients with COPD and to result in ventilatory failure (Roussos & Macklem 1977, Rochester 1981). Although of insufficient benefit to protect against fatigue development, the small effect on low frequency forces seen here may have relevance to the management of patients with COPD.

STUDY CRITIQUE

Few previous studies in man have used a double-blind randomised placebo-controlled protocol as was done in this

work. The design entailed administration of aminophylline unblind for several days before both placebo and active drug ingestion, and thereby ensured that the two limbs of the study were identical, allowed tolerance to side-effects to develop, and obscured the identity of the succeeding agent.

The technique of quadriceps stimulation was used first by Edwards and colleagues (1977a), who found that stimulation of the muscle surface activated up to 2/3 of the muscle bulk. They chose to limit the stimulated force to 1/3 of the maximum because of the potential risk of injury to the leg (Edwards et al 1977a). Thus, the resultant force is not truly representative of a maximally stimulated muscle. This might affect the response of the muscle to fatigue. Edwards and colleagues (1977a) found that partial stimulation produced similar results for the FFC and muscle relaxation rate as complete stimulation of the muscle (Edwards et al 1977a). In the present study, the mean force at 100Hz before fatigue was comparable with that reported by Edwards and co-workers (1977a): approximately 38% of MVC.

The voltage of stimulation was chosen on the basis of the MVC on each study day. This could have resulted in differing levels of stimulation, and therefore, different levels of fatigue. As the MVCs and the force at 20Hz during the first pre-fatigue FFCs did not differ on the two treatments, the levels of stimulation of the muscles on the two treatments were comparable also. Thus, the comparison between days was valid as the muscles were stimulated under the same conditions and to the same extent each day.

The differences between treatments in low frequency forces during the third FFCs were very similar for both legs. The low frequency forces for the left leg, and the 20:100Hz ratio for the right leg after fatigue were greater on theophylline than on placebo. Had the cohort been larger, the treatment differences in low frequency forces for the right leg and in 20:100Hz ratio for the left leg after fatigue might have been statistically significant also.

Another possibility is that theophylline affected nerve axon excitability, particularly at 20Hz stimulation, thereby altering the population of muscle fibres stimulated and influencing the extent of fatigue-development.

The difference in force developed during the first and third pre-fatigue FFCs and the first and third post-fatigue FFCs was not expected from preliminary investigations. The differences imply further low frequency fatigue occurred because of the FFCs themselves. In the case of the post-fatigue FFCs, low frequency fatigue also might have continued to develop after the completion of the fatigue run. The latter is possible as the first FFC was performed only 1.5 minutes after the end of the fatiguing run, whereas the third FFC was performed another 10 minutes later.

The interpretation of the differences between FFCs is difficult. The significance of the effects of theophylline on the FFCs cannot be fully determined. Despite this, theophylline did cause some attenuation of low frequency fatigue in the quadriceps femoris.

CHAPTER 4

STUDY 2

THE EFFECT OF INTRAVENOUS AMINOPHYLLINE ON BILATERAL DIAPHRAGMATIC TWITCHES IN MAN

INTRODUCTION

Previous studies of the effect of aminophylline on the transdiaphragmatic pressure developed during artificial stimulation of the diaphragm in healthy subjects have produced conflicting results. In a study by Aubier and colleagues (1981a) the frequency-force relationship of the hemidiaphragm was investigated before and after the production of low frequency fatigue, and the effect of aminophylline was noted. Following aminophylline there was a shift in the fatigued frequency-force curve to a more normal relationship with restoration of transdiaphragmatic pressure at low frequencies.

When Moxham and co-workers (1985) studied the unilateral diaphragmatic twitch, they could identify no statistically significant increase in transdiaphragmatic pressure following aminophylline infusion. The reason for this difference in findings is unclear. One explanation is that in the latter study, as the authors postulated, an increase in twitch tension of 10% or less was too small to be detected so that enhancement of the twitch was missed.

Another possibility is that the methods of producing diaphragmatic contraction lead to spurious measurements. In both studies only one hemidiaphragm was made to contract by stimulation of the ipsilateral phrenic nerve. Contraction of one hemidiaphragm may give an inaccurate assessment of diaphragmatic contraction (Bellemare et al 1986) because of paradoxical movement of the contralateral relaxed hemidiaphragm. The potential abdominal pressure increment is

dissipated by transmission to the thoracic cavity, and there is little change in transdiaphragmatic pressure. This possible confounding factor operated in both studies although it may have influenced the accurate measurement of the relatively smaller twitch tension to a greater extent.

Bilateral phrenic nerve stimulation eliminates these drawbacks. The twitch amplitude is at least twice that of a unilateral twitch (Mier et al 1985d, Bellemare et al 1986), and enhancement is more likely to be detected. The resultant bilateral diaphragmatic contraction is more representative of the diaphragm's physiological behaviour during respiration than a unilateral contraction.

Investigation of twitch transdiaphragmatic pressure is appropriate because the effect of aminophylline on skeletal muscle is reported to be due to an action on twitch characteristics (Jones et al 1982), and because achieving bilateral tetanic stimulation is difficult and unreliable. A technique first used by Maclean and Mattioni (1981) and developed by Aubier and colleagues (1985c) of bilateral phrenic nerve stimulation with needle electrodes was used.

AIMS

To investigate the effect of aminophylline on bilateral diaphragmatic twitch tension.

SUBJECTS

Four healthy non-smoking volunteers (three males) aged 30-44 years entered the study. They were from the staff of the Brompton Hospital and the Clichy Hopital in Paris. Three were

familiar with the procedures and had taken part in similar studies in the past. The fourth had no experience of such investigations. All subjects were seated comfortably in an armchair, and remained in the same position throughout the study.

METHODS

The method of phrenic nerve stimulation is described in Chapter 2, Section 1, Pages 136-138, and Section 2, Pages 150-151. Transdiaphragmatic pressure, left and right diaphragmatic electromyograms and rib cage and abdominal anteroposterior diameters were measured as described in Chapter 2, Sections 1 & 2.

PROTOCOL

Owing to the likelihood of side effects occurring and the plasma half-life of aminophylline, the study was not conducted blind, nor with randomisation of the measurement periods. The design was open, and conformed to the protocol described in Chapter 2, Section 4, Pages 174 & 176, Figure 2.13.

Subjects were asked to breathe quietly and then to relax at end-expiration with closed glottis during bilateral phrenic nerve stimulation.

Transdiaphragmatic pressure, left and right diaphragmatic electromyograms, rib cage and abdominal anteroposterior recordings were taken during a control period comprising two-minute stimulation runs performed at five-minute intervals for 20-30 minutes. After this, aminophylline was infused at

dosages of 6-7.5 mg/kg for 30 minutes. Phrenic nerve stimulation was then repeated for another 15-minute period, following the same procedure as for the control runs. At the end of this period, blood for theophylline assay was taken from the arm opposite to that through which the infusion had been given.

DATA HANDLING AND STATISTICAL ANALYSIS - ALL DATA

All twitches showing evidence on either electromyogram or transdiaphragmatic pressure traces of interference by cardiac contraction were excluded from analysis. All other twitches which appeared to lie at functional residual capacity as judged by flat and stable traces for transdiaphragmatic pressure at end-expiration, rib cage anteroposterior diameter and abdominal anteroposterior diameter were analysed. Left and right electromyogram amplitudes ie. the compound muscle action potential, were measured as the peak-to-peak distance. Twitch transdiaphragmatic pressure was recorded as the difference in the amplitude from transdiaphragmatic pressure at end-expiration to the peak of the twitch.

Unpaired t tests were used to determine statistical differences between control and aminophylline run twitches for all individual data, and two-way analysis of variance for the mean data.

Correlations were conducted between the compound muscle action potentials and transdiaphragmatic pressure at end-expiration and chest wall diameters.

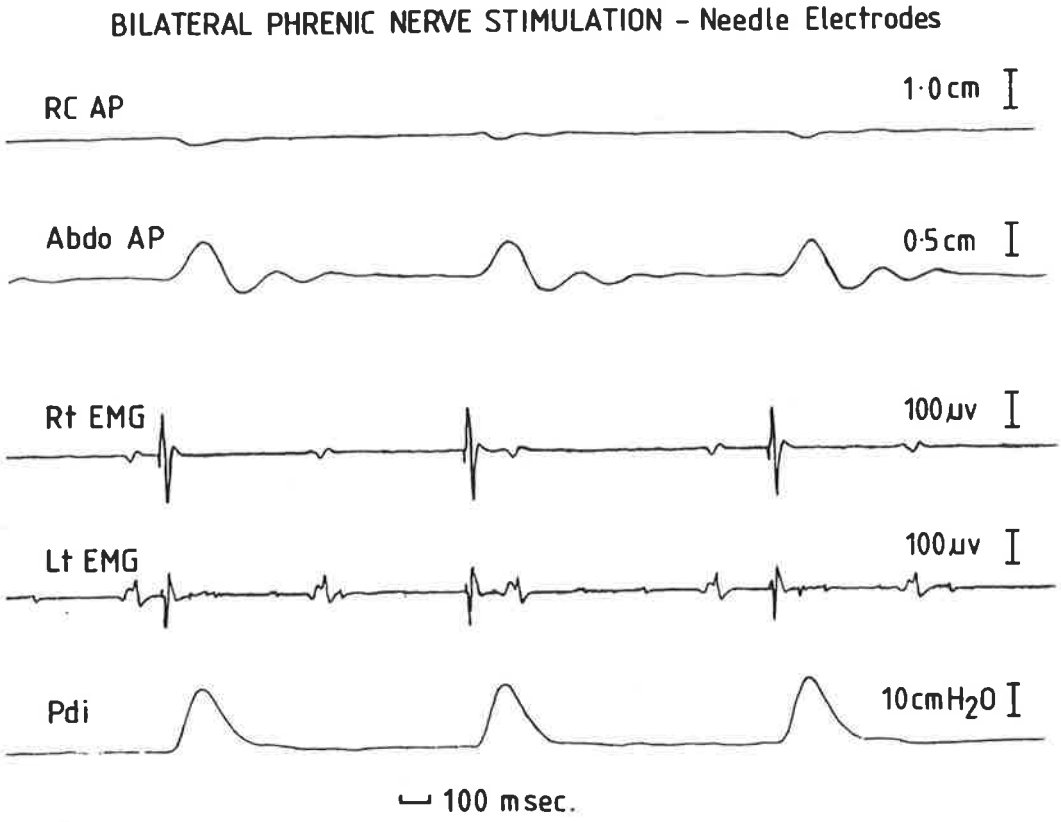
RESULTS - ALL DATA

An example of a typical trace is given in Figure 4.1.

Subjects 1, 3 and 4 had increased twitch transdiaphragmatic pressures after aminophylline, while Subject 2 had a decrease ($P<0.001$) (Table 4.1). In addition, left and right electromyogram amplitudes differed for all subjects, with variable changes between and within individuals (Figure 4.2). In the case of the parameters measured immediately prior to the twitch (ie. transdiaphragmatic pressure, rib cage and abdominal anteroposterior diameters), there were statistically significant treatment differences for Subjects 2 and 4 for transdiaphragmatic pressure and abdominal diameter at end-expiration, and for Subject 1 for rib cage diameter at end-expiration (Table 4.2). The direction of change in the variables was not consistent. For the group as a whole, there were no statistically significantly differences between the two treatment runs of twitches.

Percentage changes in twitch transdiaphragmatic pressure and left and right electromyogram amplitudes are presented in Figure 4.2. The mean difference in twitch transdiaphragmatic pressure was 5.5% and the corresponding differences in left and right electromyogram amplitudes were a mean reduction of 6.3% and a mean increase of 4.7% respectively. None of these mean differences was statistically significant.

FIGURE 4.1



A typical trace of bilateral phrenic nerve stimulation with needle electrodes from one subject, showing from the top, rib cage anteroposterior diameter (RC AP), abdominal anteroposterior diameter (Abdo AP), right diaphragmatic electromyogram (Rt EMG), left diaphragmatic electromyogram (Lt EMG) and twitch transdiaphragmatic pressure (Pdi). The compound muscle action potential can be seen on Rt and Lt EMG traces immediately before the twitch Pdi deflection. The other deflections in the EMG traces are electrocardiograms.

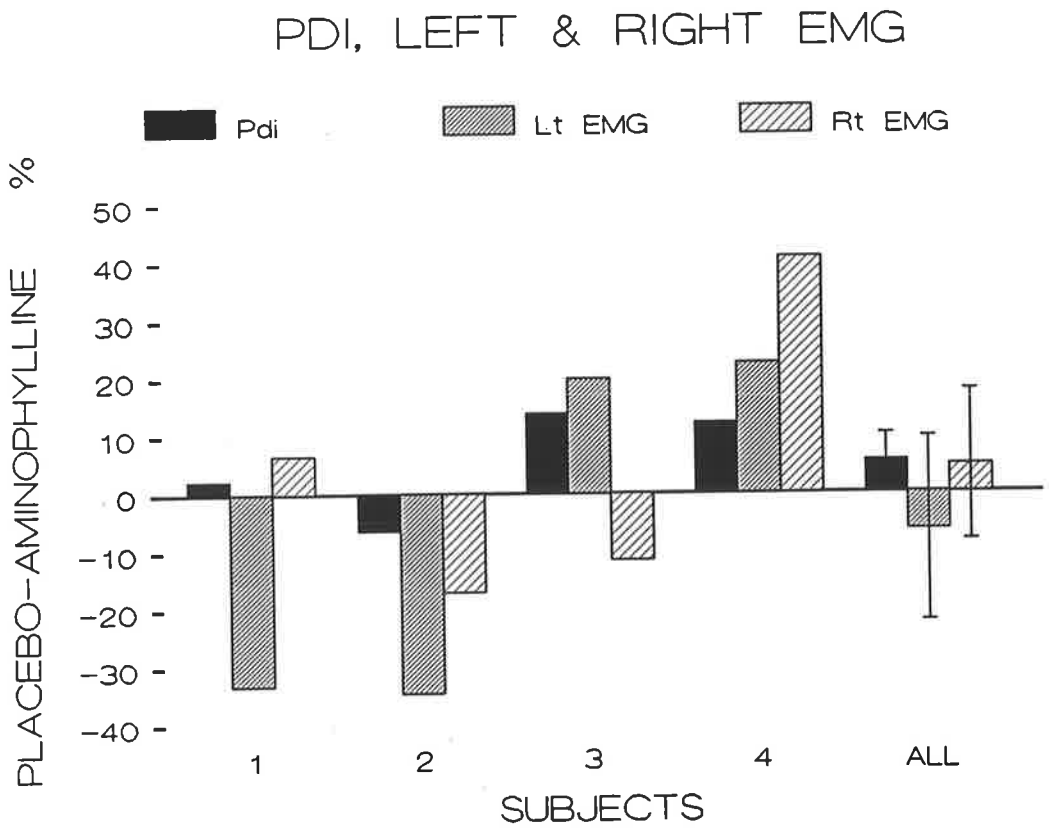
TABLE 4.1

TWITCH PDI – ALL DATA

SUBJECT No.	CONTROL cmsH2O	AMINO cmsH2O	DIFF %
	MEAN [SD]		
1	12.9 [1.5] n=120	13.2 [1.2] n=173	2.3*
2	20.6 [3.5] n=300	19.3 [1.7] n=124	-6.2*
3	17.4 [1.3] n=51	19.8 [0.9] n=83	13.8*
4	20.5 [2.5] n=72	23.0 [1.9] n=210	12.2*
MEAN (SEM)	17.8 (1.8)	18.8 (2.0)	5.5 (4.7)

Mean twitch transdiaphragmatic pressures (twitch Pdi) and standard deviation (SD) (mean & standard error of the mean [SEM] for the group) for all twitches measured at resting end-expiration. The differences between control and aminophylline values were statistically significant for all subjects (*P<0.001). Treatment differences for the group were not statistically significant.

FIGURE 4.2



The percentage differences in twitch Pdi, and left and right EMG amplitude for all data between control and aminophylline runs. Individual differences between control and aminophylline values of left and right EMG amplitudes for all subjects were statistically significant, however the group differences for both twitch Pdi and left and right EMGs did not differ statistically significantly.

TABLE 4.2

PDIFRC, RC AP, ABDO AP – ALL DATA

SUBJECT No.	PDIFRC cmsH2O	RC AP mm	ABDO AP mm
1	0.4* n=120	2.8 n=173	3.7*
2	0.4 n=300	−5.2* n=124	−1.6
3	1.9* n=51	10.6* n=83	7.2*
4	−2.5 n=72	−3.6* n=210	7.6
MEAN [SEM]	0.05 [0.92]	1.15 [3.6]	4.23 [2.11]

The mean differences between control and aminophylline values of transdiaphragmatic pressure at resting end-expiration (PdiFRC), rib cage (RC) and abdominal (Abdo) anteroposterior (AP) diameters immediately before a twitch, for all twitches (Aminophylline value - placebo value). There were no statistically significant treatment differences for the group, although differences in most variables for individuals were statistically significant (*P<0.05). The exceptions were Subjects 2 and 4 for PdiFRC and AbdoAP diameter, and Subject 1 for RCAP diameter.

DISCUSSION - ALL DATA

Comparison of individual results indicates that not only were differences in twitch transdiaphragmatic pressure between the aminophylline and control periods variable in terms of degree and direction (Table 4.1), but so were the differences in left and right electromyogram amplitudes (Figure 4.2), transdiaphragmatic pressure and rib cage and abdominal anteroposterior diameters at end-expiration (Table 4.2). In addition, some of the differences were relatively large. This makes the interpretation of the changes and the identification of a genuine effect on the diaphragm problematic.

The large differences in electromyogram amplitudes between measurement periods could be due to a number of causes. These include a shift in the relative position of the stimulating electrodes, a change in the resistance of the tissues around these electrodes or beneath the recording electrodes, and movement of the diaphragm relative to the recording electrodes, so that the strength of the electromyogram signals detected by the recording electrodes altered between the two measurement runs. The fact that the changes in left and right electromyogram after aminophylline infusion differed in degree and, for Subjects 1 & 3, in direction suggest they could not have been due solely to aminophylline or to any other single cause.

In support of this view is the fact that there were small differences in transdiaphragmatic pressure at end-expiration and in the chest-wall dimensions, which together may indicate

alterations in the configuration or length of the diaphragm, sufficient to affect its contractility.

In order to reduce the influence of these factors on the data and to assist the interpretation of the study selected twitches were also analysed. Selected twitches during the two runs were matched as closely as possible with respect to all parameters but twitch transdiaphragmatic pressure.

DATA HANDLING AND STATISTICAL ANALYSIS - SELECTED TWITCHES

Initially, the traces of the rib cage and abdominal anteroposterior diameters and transdiaphragmatic pressure at resting end-expiration were measured immediately before each twitch and the left and right electromyogram amplitudes were also measured. The mean and standard deviation (SD) of these parameters were determined separately for the control and for the drug runs.

The selected twitches were defined as those with any three or more of these variables lying in a range common to both measurement periods. The ranges were within two SD of the means for each period. This selection criterion was imposed because it defined the normal range of these parameters.

Unpaired t tests were used to determine statistical differences between selected twitches during the control and aminophylline runs for all individual data, and two-way analysis of variance for the mean data.

RESULTS - SELECTED TWITCHES

The results for selected twitches are presented in Tables 4.3 and 4.4 and Figure 4.3.

TABLE 4.3

TWITCH PDI – SELECTED DATA

SUBJECT No.	CONTROL cmsH2O	AMINO cmsH2O	DIFF %
	MEAN [SD]		
1	12.3 [1.5] n=36	13.2 [1.3] n=96	7.5*
2	22.8 [3.0] n=47	19.2 [1.8] n=77	-15.8*
3	18.2 [0.6] n=6	19.6 [0.8] n=36	7.7*
4	20.2 [1.3] n=18	22.7 [2.2] n=70	12.4*
MEAN (SEM)	18.4 (2.2)	18.7 (2.0)	3.0 (6.4)

Mean [SD] twitch transdiaphragmatic pressures (twitch Pdi) for selected twitches measured at resting end-expiration. Control and aminophylline values for all subjects differed statistically significantly (*P<0.001). The group control and aminophylline values (mean & SEM) were not statistically significantly different.

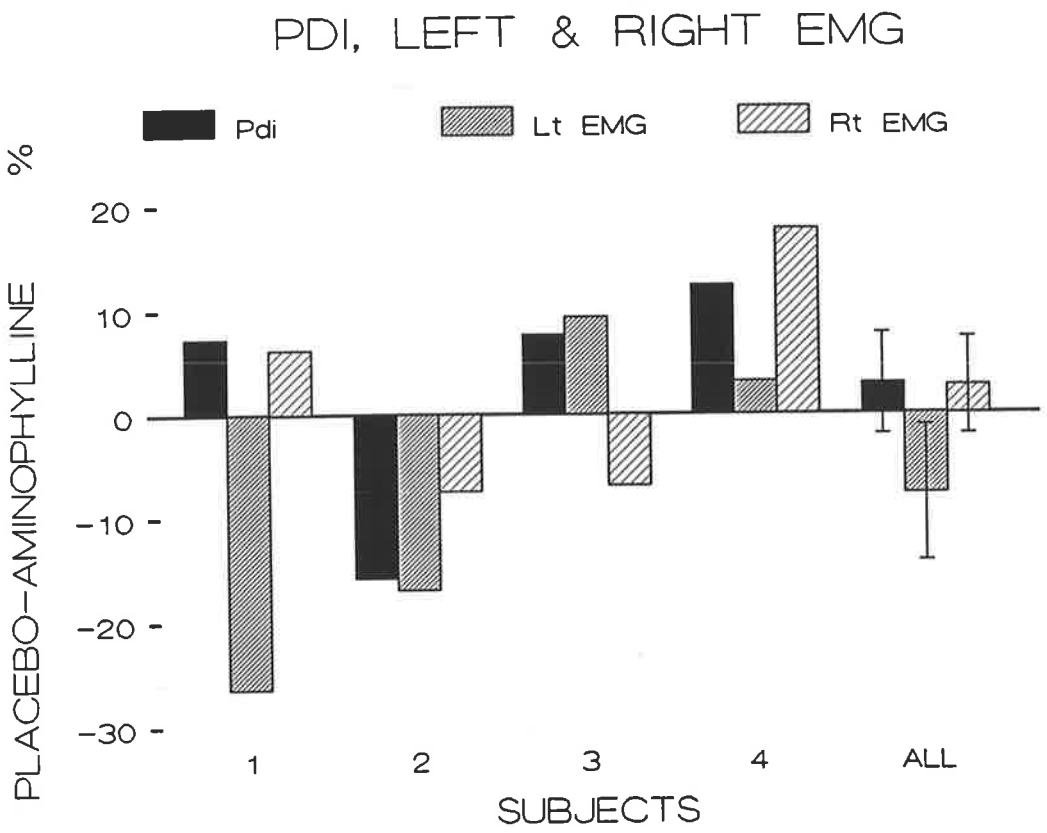
TABLE 4.4

PDIFRC, RC AP, ABDO AP – SELECTED DATA

SUBJECT No.	PDIFRC cmsH2O	RC AP mm	ABDO AP mm
1	0.4* n=36	0.6 n=96	2.2*
2	−0.4 n=47	5.2* n=77	0.3
3	0.9* n=6	10.8* n=36	7.0*
4	−0.4 n=18	−7.2* n=70	0.5
MEAN [SEM]	0.125 [0.32]	2.05 [3.86]	2.45 [1.55]

The mean differences between control and aminophylline values of transdiaphragmatic pressure at resting end-expiration (PdiFRC), rib cage (RC) and abdominal (Abdo) anteroposterior (AP) diameters immediately before a twitch (Aminophylline value - placebo value). The group differences (mean & SEM) were not statistically significant, however, individual differences for some parameters were (*P<0.05)). The exceptions were Subjects 2 and 4 for PdiFRC and AbdoAP diameter, and Subject 1 for RCAP diameter.

FIGURE 4.3



The percentage differences (mean & SD) in twitch Pdi, and left and right EMG amplitude for selected twitches. The group results (mean & SEM) did not differ statistically significantly, although all individual treatment differences were statistically significant.

Subjects 1, 3 & 4 had increases in twitch transdiaphragmatic pressure after aminophylline of 7%, 8% and 12% respectively, and subject 2 showed a decrease of 16% ($P<0.001$) (Figure 4.3). The corresponding differences in left and right electromyogram ampiltudes after aminophylline compared with before were -26% and 6%, 9% and -7%, 3% and 18% respectively for Subjects 1, 3 and 4, and -17% and -8% respectively for Subject 4 ($P<0.001$) (Figure 4.3).

The differences between aminophylline and control values for mean twitch transdiaphragmatic pressure and mean left and right electromyogram amplitudes were 2.9%, -7.7% and 2.4% respectively. Transdiaphragmatic pressure at end-expiration differed by less than 1cmH_2O (mean 0.1cmH_2O) and rib cage and abdominal anteroposterior diameters by no more than 1.1cm (mean 0.2cm) and 0.7cm (mean 0.2cm) respectively for any subject between the control and aminophylline runs of selected twitches (Table 4.4).

Theophylline levels were all within the therapeutic range. The mean $\pm$ was 13.8mg/l $\pm$1.5, range 12.0-15.0mg/l.

One subject (No. 3) complained of severe shaking and nausea, and had obvious tremor after aminophylline. The other subjects were also aware of feelings of shakiness, but had no signs.

DISCUSSION

Bilateral phrenic nerve stimulation allows investigation of the diaphragm alone, without the activation of other muscles, and without depending on subject cooperation (Bellemare &

Bigland-Ritchie 1984, Gandevia & McKenzie 1985).

The effect of methylxanthines is thought to be relatively greater on the twitch than on contractions produced at higher frequencies of stimulation (Jones et al 1982). Thus, the bilateral twitch should prove a favourable parameter for the detection of an inotropic action by the drug. Even so, the overall increase in twitch height with aminophylline was 2.9%, and this small change was not statistically significant.

A mean increase in twitch transdiaphragmatic pressure of 2.9% is considerably less than that reported recently by Murciano et al (1987), but is in agreement with those of Moxham and colleagues (1985) and Levy et al (1990).

Using unilateral phrenic stimulation, Moxham and colleagues (1985) noted a small (4%) effect of aminophylline on twitch tension, which was also not statistically significant. These authors stressed that their findings did not preclude an increase in twitch amplitude of up to 10% with aminophylline, the size of unilateral twitches making a change smaller than 10% unreliable to detect. The size of bilateral twitches, more than twice that of unilateral, allows relatively smaller changes in twitch height to be measured, providing greater confidence in the results.

In the current study, and studies by Murciano and colleagues (1987) and Levy and co-workers (1990), the action of aminophylline on the bilateral diaphragm twitch was studied. Murciano et al (1987) reported an improvement in twitch transdiaphragmatic pressure of 23%, while Levy and co-workers (1990) reported a small, statistically insignificant increase

of 3.7% in mean bilateral twitch tension.

It is difficult to determine reasons for the discrepancy between the positive results (Murciano et al 1987) and those of both the present study and the work by Levy et al (1990). In the study by Levy and colleagues (1990), right electromyogram amplitude increased by 8%, while left electromyogram amplitude, chest wall dimensions and gastric pressure did not change after aminophylline.

From the data available in the report by Murciano et al (1987), it cannot be determined whether or not small changes occurred in lung volume, diaphragmatic length and configuration, which may have affected diaphragmatic contractility. In addition, it is possible that the diaphragm might have contracted isometrically at end-expiration during the course of the study, producing a change in transdiaphragmatic pressure prior to the twitch, and so resulting in an increase in twitch tension (De Troyer & Martin 1983). This would not necessarily have resulted in an increase in the compound muscle action potential as diaphragmatic activation would not have altered. Indeed, the electromyogram amplitudes did not differ between treatment periods. If resting diaphragmatic tension was affected, the inotropic effect of aminophylline on twitch tension might have been overestimated. It is also possible that intercostal activity might have resulted in larger oesophageal pressure swings than seen with solitary diaphragmatic contraction.

In the current study, the influence of other inspiratory

muscles on the measured twitch was minimal as phrenic nerve stimulation occurred at end-expiration when the muscles of the chest wall were relaxed. This is supported by the electromyogram record which showed no contamination of the compound muscle action potentials and no activity between twitches, implying relaxation of the diaphragm and the intercostal muscles beneath the recording electrodes. In addition, gastric pressure at end-expiration and the abdominal anteroposterior diameter differed minimally (<3cmH_2O & 0.7cm respectively) between the control and aminophylline runs, suggesting that the abdominal muscles did not cause the differences in diaphragmatic twitch tension between the two treatment periods (Levy et al 1990).

STUDY CRITIQUE

Analysis of all data showed small individual changes in twitch transdiaphragmatic pressure from the control to the aminophylline periods (Table 4.1). However, these changes were accompanied by inconsistent changes in left and right electromyogram amplitudes (Figure 4.2), and slight, inconsistent, changes in chest-wall dimensions and transdiaphragmatic pressure at end-expiration (Table 4.2), between the two study periods.

The selected twitches more closely matched for variables influencing diaphragmatic contractility, than was the case when all data were analysed. The actual differences between values of transdiaphragmatic pressure, rib cage and abdominal anteroposterior diameters were very small.

Rather than producing bias, this selection process thus makes simpler the interpretation of changes in twitch transdiaphragmatic pressure (Konno & Mead 1967, Grassino et al 1978, Kim et al 1985, Gandevia & McKenzie 1986). Konno and Mead (1967), and Grassino et al (1978) found that there is a correlation between abdominal and rib cage dimensions, diaphragmatic configuration and contractility. The selection of twitches at similar rib cage and abdominal anteroposterior diameters and transdiaphragmatic pressure at end-expiration allowed diaphragmatic contractility at similar diaphragmatic length and configuration, as well as lung volume, to be compared.

There was also greater similarity of individual left and right electromyogram amplitudes, except for Subject No. 1, in the two treatment runs, implying greater similarity of diaphragmatic activation for selected twitches than for all data. Electromyogram amplitude is known to alter with lung volume and posture, so that matching transdiaphragmatic pressure and chest wall dimensions immediately prior to the twitch for pre- and post-aminophylline twitches eliminates variables which influence electromyogram amplitude as well as twitch transdiaphragmatic pressure (Kim et al 1985, Gandevia & McKenzie 1986).

Despite this, it is possible that the effect of aminophylline on mean twitch transdiaphragmatic pressure was under-estimated as there were substantial changes in electromyogram amplitudes. Although, as explained in Chapter 1, Section 5, Pages 121-123, the relationship between

transdiaphragmatic pressure and compound muscle action potential is not known, the electromyogram does reflect phrenic nerve activation. The large falls in left electromyogram amplitude for Subject 1 and 2 and the smaller falls in right electromyogram amplitude for Subjects 2 and 3 after aminophylline, might indicate that muscle activation had also decreased, effectively masking potential increases in twitch tension due to the drug.

Less effective phrenic nerve stimulation might have occurred in these subjects, due perhaps to slight movement of the stimulating electrodes, or to local oedema. Care was taken to minimise such occurrences. For each subject, the stimulating electrodes were secured firmly in place once the position in the neck of maximal phrenic nerve stimulation was determined. The voltage was increased until the compound muscle action potential for each side and twitch transdiaphragmatic pressure no longer increased, and was kept above this level. Only then were control twitches recorded. During the study, the voltage was increased two or three times to ensure that the voltage producing maximal stimulation was unchanged.

Another possible explanation for the alterations in electromyogram amplitude is that the contracting diaphragm moved relative to the recording electrodes. The subjects were seated comfortably in an armchair, and remained still throughout the study, in order to minimise posture changes. The closely matched values for transdiaphragmatic pressure and rib

cage and abdominal anteroposterior diameters for all subjects during the two measurement periods suggest that posture changes were very small, so that this explanation seems unlikely.

The sensitivity of the recording electrodes might have been sufficiently great to produce marked differences in electromyogram amplitudes within the range of chest wall and diaphragm movement. This did not seem to be the case from inspection of the traces. Correlations conducted between left or right compound muscle action potential and transdiaphragmatic pressure at end-expiration or chest wall diameters failed to identify any correlations between EMG amplitudes and chest-wall movement. In addition, the extent and direction of change in left and right electromyogram amplitudes were variable within each individual.

A combination of all of these factors might have been responsible for the alterations in compound muscle action potential amplitude. Slight changes in the level of activation of the nerve may have occurred; while in addition, alteration in resistance in the tissues underlying the recording electrodes, or movement of the diaphragm relative to the electrodes may have resulted in a decreased or increased electromyogram. Alternatively the differences in electromyogram amplitude may relate to an effect of aminophylline within the axon or neuromuscular junction, changes which may have also influenced the twitch tension.

Irrespective of the causes, the changes in electromyogram amplitudes make the effect of aminophylline on twitch

transdiaphragmatic pressure difficult to interpret.

Aubier and colleagues (1981a) and Supinski and co-workers (1984a) have expressed the effect of aminophylline in terms of the relationship between transdiaphragmatic pressure and the sum of the left and right compound muscle action potential amplitudes ie. the so-called Pdi:Edi ratio. They concluded that there was an increase of 15% after theophylline. The existence of such a Pdi:Edi relationship was tested for each individual. No consistent correlation was detected. For this reason and those given in Chapter 1, Section 5, Pages 121-123, the procedure has not been adopted here.

In addition, recent work has shown that, even if maximal activation of both hemidiaphragms is not achieved during bilateral stimulation, a maximal diaphragmatic contraction can still result, as long as one hemidiaphragm is maximally activated and the other contracts sufficiently to prevent the chest wall distortion that will otherwise occur (Bellemare et al 1986, Nava et al 1987). Thus a failure of complete phrenic nerve stimulation on one side with reduction in the electromyogram, may not necessarily imply failure of ipsilateral diaphragmatic activation, nor a falsely low twitch transdiaphragmatic pressure.

The mean decrease in left electromyogram amplitude of 7.7% in this study, might have been due to decreased phrenic nerve stimulation. Had the amplitude remained constant, it is possible that twitch transdiaphragmatic pressure might have increased by more than was seen. The extent of the further

increase in twitch tension cannot be judged from current knowledge and the data available.

The major technical problem with this study relates to the alterations in electromyogram amplitudes, and the difficulty in interpreting the reasons for the changes, and the consequent effect on twitch transdiaphragmatic pressure. There are insufficient data to make these interpretations. Greater subject numbers might have helped the interpretation, although the greatest benefit would have arisen from being able to match left and right electromyograms exactly during aminophylline and control twitches in each individual.

Conducting the study with subjects supine, and with the head and stimulating electrodes in a brace, as used by Gandevia and McKenzie (1985) might have reduced the variability in compound muscle action potential amplitude.

In other respects the study provided useful information. It was the first study of the effect of aminophylline on the bilateral diaphragmatic twitch (Brophy et al 1985b). It is the only work to match twitches for transdiaphragmatic pressure, rib cage and abdominal anteroposterior diameters at end-expiration, and thereby to ensure that diaphragmatic length and configuration and lung volume were constant during the two treatment periods.

CHAPTER 5

STUDY 3

THE EFFECT OF INTRAVENOUS AMINOPHYLLINE ON THE PACED DIAPHRAGM IN QUADRIPLEGIC PATIENTS

INTRODUCTION

Phrenic nerve pacing to achieve diaphragmatic contraction has been used for several years in the long term management of quadriplegic patients requiring ventilatory support (Glenn et al 1972, 1980, 1986). Continuous pacing of one or both hemidiaphragms has been reported to result in diaphragmatic fatigue (Oda et al 1981). Pacing of one hemidiaphragm for periods of 12 hours or less allows time for the diaphragm to rest, and this, combined with the use of low frequencies and short pulse-widths for pacing, has reduced the risk of fatigue.

Current practice is that pacing is initiated for several minutes only and the duration gradually extended until each hemidiaphragm is paced for several hours at a time. Nochomovitz and colleagues (1984) and Garrido et al (1987) reported patients who, after receiving a conditioning regimen of phrenic nerve pacing, could be continuously ventilated with pacing alone. In addition, Nochomovitz et al (1984) found that aminophylline improved diaphragmatic contraction in the short term, and believed that it helped to prevent the development of fatigue.

In another case, reported by Chevrolet and co-workers (1983), aminophylline was given to a patient with a unilateral phrenic nerve pacer following failure of effective ventilation after bilateral chordotomy. There was improvement in both spontaneous and paced ventilation. The authors concluded that the beneficial effect of aminophyline was due primarily to stimulation of ventilation centrally via O_2 chemoreceptors, but

that a direct effect on diaphragmatic contractility may have resulted also.

As patients with high transections of the cervical cord have complete disruption of central innervation to the diaphragm, those with phrenic nerve pacers provide a means of determining the direct effect of a drug on the muscle alone.

AIMS

Two quadriplegic patients with phrenic nerve pacers were assessed in order to determine the direct effect of aminophylline infusion on the centrally denervated diaphragm.

PATIENTS

PATIENT 1

A 21 year-old man suffered a fracture-dislocation of the first and second cervical vertebrae as a result of a motor-cycle accident. Prior to the accident he was completely well and had no history of respiratory disease. Mechanical ventilation via a tracheostomy was initiated and he was maintained on this for several months. However, due to the fact that a sufficient tidal volume could not be sustained whilst he was seated upright, he was referred for implantation of phrenic nerve pacers. There was no recovery of his complete cord transection apart from the return of very slight sensation in the third cervical nerve root dermatome.

In order to assess his ventilatory capacity and diaphragmatic function, he was disconnected from the ventilator on two occasions, once soon after the injury and a second time seven months later, before the implantation

operations. On the first occasion, he achieved a tidal volume of 100 ml using the accessory muscles of respiration, but this was not sustained, and the second time he was able to produce a tidal volume of nearly 500mls for a few breaths by contraction of the sternomastoid muscles. No diaphragmatic contraction occurred.

Prior to operation, the viability of the phrenic nerves was determined by bilateral transcutaneous phrenic nerve stimulation in the neck, and by fluoroscopic screening for diaphragmatic movement, while disconnected from the ventilator (Lozewicz et al 1981). Both nerves were intact.

Two operations, one week apart, were performed to implant monopolar electrodes close to the phrenic nerve in the neck on the right and in the thorax on the left. A radio-receiver was implanted superficially into the soft tissue of the chest-wall on each side. Post-operative diaphragmatic screening showed that pacing produced good movement of both hemidiaphragms, and that maximum tidal volumes of 750ml and 700ml respectively were achieved with right and left diaphragm pacing via the tracheostomy, without accessory muscle contraction. Pacing of each side was initiated separately for ten minutes every hour (Glenn et al 1972, 1986).

PATIENT 2

This patient, a 54 year-old man, had no history of respiratory disease and, five years previously, had sustained a spinal cord injury at the level of the first and second cervival vertebrae. At the time of the injury he was able to

produce a tidal volume of 500 mls for a short period by contraction of his sternomastoid muscles alone, but for long term management required mechanical ventilation via a tracheostomy. No voluntary or spontaneous diaphragmatic contraction was detected.

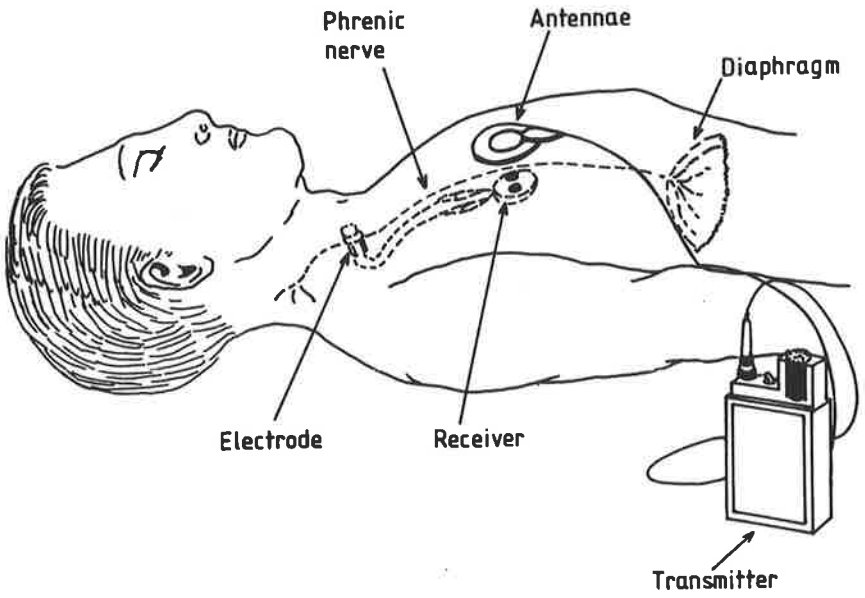
Following the insertion of bilateral phrenic nerve pacers, five years before this admission, he was successfully managed without a tracheostomy with 24 hour phrenic nerve pacing by the technique of Glenn and colleagues (1980, 1984). He was followed with yearly pacer checks in hospital, the occasion of this study coinciding with such a routine admission.

METHODS

The phrenic nerve pacing electrodes operate through an electromagnetic signal transmitted from a small battery-operated pacing box via an extracorporeal antenna placed over the implanted radio-receiver (Figure 5.1). The electrodes themselves are placed close to but not touching the phrenic nerve in the neck on one side and in the thorax on the other (Lozewicz et al 1981).

Respiration during pacing results from delivery of a series of impulses to the phrenic nerve. The square wave impulses have a pulse width of 0.1msec and a pulse interval of 400msec, and are applied at 25Hz for 1.3 seconds. They are timed to produce 12 breaths a minute and the voltage of stimulation is ramped in order to mimic physiological phrenic nerve traffic. Using this method of impulse delivery the risk of neuromuscular failure or muscle fatigue is minimised (Glenn et al 1972).

FIGURE 5.1



The method of phrenic nerve pacing. An antenna placed over the implanted radio-receiver transmits the pacing signal from the transmitter box. The electrodes are implanted to lie adjacent to the phrenic nerve.

Adapted from Diaphragm Pacer Applications Manual, Avery Laboratories, Inc. USA.

Left and right diaphragmatic electromyograms, rib cage antero-posterior, rib cage lateral and abdominal antero-posterior diameters and, in the case of Patient 1, transdiaphragmatic pressure, were measured and recorded as described previously in Chapter 2, Sections 1 and 2. The radio-frequency output of the pacing transmitter was recorded with a transistor radio-receiver by suspending the antenna over the implanted radio-receiver.

Maximum relaxation rate of transdiaphragmatic pressure was measured on the third study day in Patient 1 (Wiles et al 1979b). Tidal volume was intermittently monitored with a Wright spirometer, either connected to the endotracheal tube in the case of Patient 1, or directly at the mouth in the case of Patient 2. For Patient 1, the volume was visually noted and manually recorded, while for Patient 2 the spirometer was connected directly to a computer for automatic read out. In the study of Patient 1 on the third measurement day, tidal volume was recorded at the start and end of each pacing period.

PROTOCOLS

The studies conformed to the design described in Chapter 2, Section 4, Pages 174 & 176, Figure 2.13.

PATIENT 1

On three occasions during the first days of pacing the patient was studied before and after an infusion of aminophylline. Left-sided pacing was studied on the first and third days, and right-sided pacing was investigated on the second day. The control and aminophylline runs were always the

first and second pacing periods of the day.

During the measurement periods the cuffed tracheostomy tube was occluded for a single paced breath every 30 seconds for 15 minutes. After the control study period, the patient was artificially ventilated and the diaphragm thereby rested for 30 minutes. An infusion of intravenous aminophylline was given over this time and blood for theophylline assay subsequently taken from the opposite arm.

Measurements were repeated during a further 15-minute period of diaphragm pacing. The left hemidiaphragm was first studied using this protocol. A week later, the right hemidiaphragm was studied during 10-minute pacing periods. Four days later, the left side was restudied with a sequence of two control runs of 15 minutes, interrupted by 30 minutes on the ventilator. An infusion of aminophylline given during a further 30-minute period of mechanical ventilation was followed by a third pacing run of 15 minutes. In each case the transdiaphragmatic pressure sustained for one second during the inspiratory phase of an occluded breath was measured.

PATIENT 2

The patient was studied on a single day. Two runs of 30 paced breaths were recorded during right and then left hemidiaphragm pacing, after which an infusion of aminophylline was given over 45 minutes. Following this two further runs of breaths during pacing of each side were recorded.

STATISTICAL ANALYSIS

For Patient 1, the best 10 measurements for each period on the first and third study days were measured. On the second day, only a short period of pacing was monitored, although the length of the pacing period was 10 minutes on each side, and hence, only five measurements during the control and aminophylline runs were recorded. Measurements on Patient 1 made on each day were compared with the paired t test. For Patient 2, all 30 measurements for each period were used. Mean data for the control and aminophylline runs were compared using the paired t test.

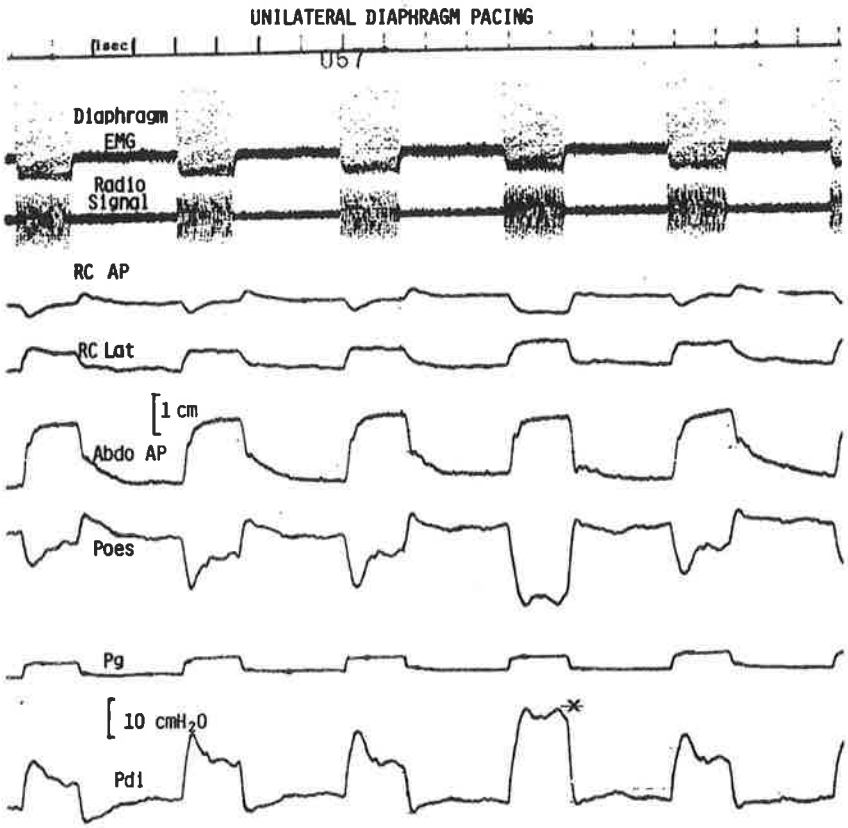
RESULTS

PATIENT 1

A typical trace during diaphragmatic pacing is shown in Figure 5.2. Transdiaphragmatic pressure fell within the first minutes of diaphragmatic pacing for each of the pacing runs. This change was due entirely to a fall in oesophageal pressure and was accompanied by an increase in lateral rib cage excursion during each paced breath. There was no change in movement of the other two chest wall dimensions.

Although tidal volume fell slightly from the beginning to the end of the pacing runs, this was not statistically significant, and did not correspond temporally to the initial fall in transdiaphragmatic pressure.

FIGURE 5.2



A typical trace during phrenic nerve pacing in Patient 1. From the top is displayed diaphragmatic electromyogram (EMG), the radio signal from the external radio-receiver representing the output from the transmitter, rib cage anteroposterior (RC AP), rib cage lateral (RC Lat), abdominal anteroposterior (Abdo AP), oesophageal pressure (Poes), gastric pressure (Pg) and transdiaphragmatic pressure (Pdi). The contraction marked with an asterisk is an occluded breath.

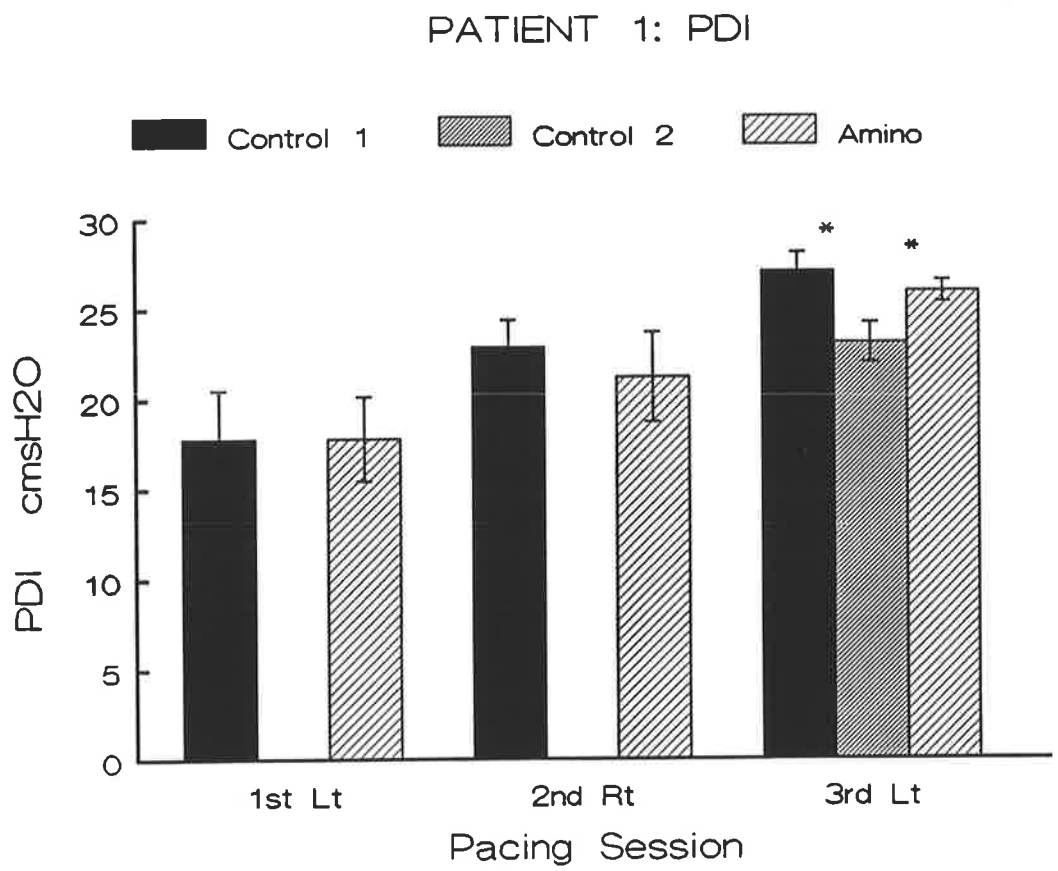
Transdiaphragmatic pressure values during pacing are presented in Figure 5.3. On the first day, mean transdiaphragmatic pressure was 17.8cmsH_2O for both the control and the aminophylline pacing runs. On the second day, mean transdiaphragmatic pressure was 22.9cmsH_2O for the control period, and 21.2cmsH_2O after aminophylline.

On the third day, two control pacing runs were performed. The mean transdiaphragmatic pressure was 27.5cmsH_2O for the first session, and 23.1cmsH_2O for the second session. Following aminophylline the mean transdiaphragmatic pressure was 26cmsH_2O, giving an increase of 12.6% on the second control session.

Tidal volumes showed similar trends. On the third day, tidal volume fell by 14.4% (from 730ml to 625ml) during the second control run, and after the spell on artificial ventilation immediately after aminophylline infusion was still 6.2% less than that at the start of the second control session (685ml compared to 730ml). By the end of the aminophylline pacing period the volume had fallen by a further 10.9% (from 685ml to 610ml).

The difference between transdiaphragmatic pressures during the control and aminophylline periods was not statistically significant when all days were analysed. However, when the data from the third day were analysed separately, mean transdiaphragmatic pressure for the the second control period was less than values for both the first control period ($P<0.05$), and for the post-aminophylline period ($P<0.001$).

FIGURE 5.3



The mean and standard deviation (SD) for transdiaphragmatic pressure (Pdi) during control and aminophylline runs on the three measurement days. On the third day two control pacing periods were performed. The subsequent aminophylline Pdi values were statistically significantly greater than the second control period values, which were also significantly less than those for the first control period (*p<0.05). Values for the first control period and aminophylline period did not differ.

The maximum relaxation rate for the third day was constant throughout the pacing sessions (Mean [SD] first control session: 8.4 [0.3]% plateau loss/10msec, second control session: 8.5 [0.9]% plateau loss/10msec, aminophylline session: 8.1 [0/7]% plateau loss/10msec.)

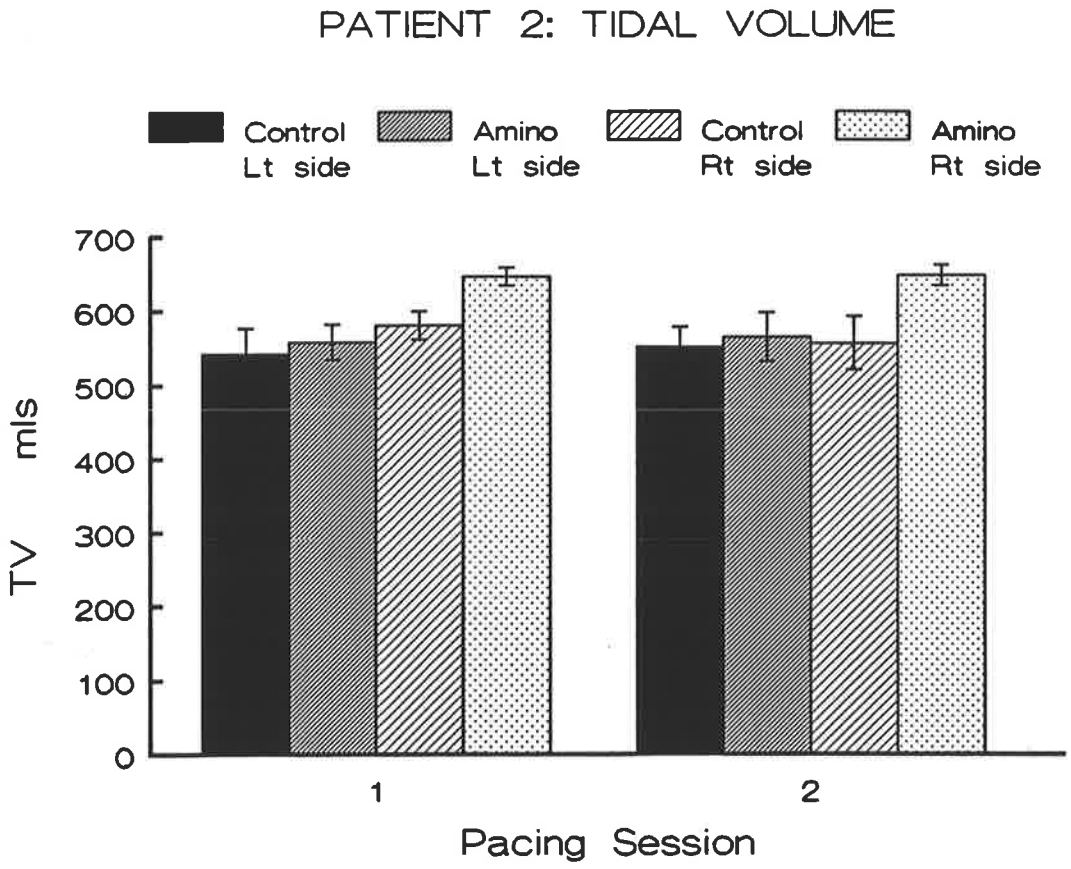
The radio-receiver signal and the electromyograms remained unchanged throughout the measurement periods, indicating that phrenic nerve stimulation and diaphragmatic activation were constant. Lung volume and the configurations of the chest wall and diaphragm at end-expiration, as judged by magnetometer and gastric pressure records did not differ between control and aminophylline periods.

Serum theophylline levels were 10mg/l on day 1, 13.4mg/l on day 2 and 15mg/l on day 3. No side effects were reported.

PATIENT 2

Tidal volumes during pacing are presented in Figure 5.4. For the control periods, tidal volumes with left side pacing were 580mls and 556mls, and with right side pacing were 542mls and 552mls. The corresponding volumes after aminophylline were 646mls and 648mls for left side pacing, and 558mls and 565mls for right side pacing. The difference between control and aminophylline periods was not statistically significant.

FIGURE 5.4



Mean and SD of tidal volumes achieved with phrenic nerve pacing before and after aminophylline infusion by Patient 2. Each of two measurement sessions for left and right side pacing before and after aminophylline are presented.

There was no difference in the electromyograms before and after aminophylline. The serum theophylline level reached after aminophylline infusion was 13.4 mg/l. The patient did not complain of side effects.

DISCUSSION

For Patient 1, during both control and drug treatment periods, shortly after the change from mechanical to pacing ventilation, there was a fall in transdiaphragmatic pressure which was due entirely to a fall in oesophageal pressure. Gastric pressure developed for each breath remained constant and abdominal wall diameter and motion was unaltered, so that diaphragmatic contraction must also have remained unchanged. The fall in oesophageal pressure was accompanied by an increase in the lateral rib cage excursion, suggesting that the rib cage had become easier to move, and possibly related to the change-over from mechanical to pacing ventilation. The fact that the slight falls in tidal volume from the beginning to the end of the pacing runs were not coincident with the change in transdiaphragmatic pressure supports this hypothesis.

This pattern was not seen for the second patient, who was paced continuously and did not require mechanical ventilation. Rib cage and abdominal motion remained constant for each breath within each study period.

Studies on Patient 1 were performed within a few days of phrenic nerve pacer implantation when the diaphragm was being conditioned to the pacing regimen, and when it is thought to be particularly susceptible to fatigue (Glenn et al 1984,

Nochomovitz et al 1984). After months of disuse the diaphragm atrophies, as do other unused skeletal muscles, and it requires conditioning to enable it to function optimally again (Glenn et al 1972, 1980, 1984). A regimen of gradually extended pacing periods is advocated to ensure that diaphragmatic function returns to normal and that diaphragmatic fatigue does not occur (Glenn et al 1972, 1984, Nochomovitz et al 1984).

Nochomowitz and colleagues (1984) reported that the force of paced diaphragmatic contraction gradually increased over several weeks with a programme of gradually extending pacing sessions. At the end of sixteen weeks of conditioning, when 24-hour continuous pacing was achieved, aminophylline was given to assess the improvement in diaphragmatic contractility. An increase in transdiaphragmatic pressure was seen at several stimulation frequencies (Nochomovitz et al 1984).

In the case of our first patient the diaphragm had not been through this conditioning schedule. Aminophylline infusion always followed the control studies because of the long half-life of the drug and the need to keep the time the patient received instrumentation to a minimum. It is possible, therefore, that a fall in transdiaphragmatic pressure during the second stimulation period was masked by an inotropic effect of aminophylline.

This appears to have occurred. The decrease in transdiaphragmatic pressure from the first to the second control run on the third day indicates that diaphragmatic fatigue did occur, despite the short pacing periods. The highest transdiaphragmatic pressures were invaribly recorded

early in a pacing run. The fall in tidal volume from the beginning to the end of the second control pacing sessions was consistent with this. The increased values that were subsequently measured during the post-aminophylline period on the third day suggest that the drug helped to reverse fatigue, although not completely. The smaller fall in tidal volume that occurred during the aminophylline pacing session supports this view.

The lack of difference between transdiaphragmatic pressures during the control and aminophylline runs on the preceding two study days might have been due to the partial prevention of threatened fatigue by the drug, or to lack of effect.

Patient 2 had been paced continuously for several years before this investigation. Diaphragmatic fatigue was not a risk. The lack of effect may be due to the fact that any inotropic effect is relatively less on fresh than fatigued muscle, and that in this case the diaphragm was not fatigued (Jones et al 1982).

In both patients, chest wall diameters at end-expiration remained the same throughout the pacing periods. The electromyogram recordings were, therefore, unaffected by changes in lung volume or diaphragmatic length (Kim et al 1985, Gandevia & McKenzie 1986). The constant electromyogram amplitudes from the control to the aminophylline runs, indicate that aminophylline had no effect on the level of phrenic nerve stimulation, or on the neuromuscular junction.

STUDY CRITIQUE

As this study involved only two patients the results are anecdotal. In addition, only one patient had transdiaphragmatic pressure measurements, which further limits the power of the work.

Nevertheless, quadriplegic patients provide a powerful model in which to test the effect of a drug on the diaphragm in isolation. The high cord lesion results in complete loss of central nervous system innervation to the intercostal, parasternal and abdominal muscles as well as to the diaphragm. However, the phrenic motor neurones, phrenic nerves and diaphragm are intact. Assuming normal lung mechanics, any effect on transdiaphragmatic pressure can be attributed to an action of aminophylline on the neuromuscular junction or on the muscle itself (Estenne et al 1980).

An additional difficulty with the study was occasioned by the open, uncontrolled design. Patient 1 was not studied under the same conditions during the control and aminophylline periods. Aminophylline always followed a control period, which was itself the first pacing session of the day. Thus, the control measurements were invariably made on a fresh, rested diaphragm, while the aminophylline measurements were made after the diaphragm had been contracting for a short time and after it may have become fatigued.

This is suggested by the findings on the third day, when the transdiaphragmatic pressure values for the second control run were statistically significantly less than those for the first control run. Although anecdotal, the improvement in

transdiaphragmatic pressures with aminophylline on this single occasion does suggest a small but genuine effect on diaphragmatic contractility.

CHAPTER 6

STUDY 4

THE EFFECT OF ORAL AMINOPHYLLINE ON THE STRENGTH OF THE RESPIRATORY AND QUADRICEPS FEMORIS MUSCLES

INTRODUCTION

Enhancement of respiratory muscle strength increases force reserve, and decreases the likelihood of fatigue development in patients who require high pressure output from the respiratory muscles (Roussos et al 1979, Bellemare & Grassino 1983). Although *in vitro* studies have shown improvement in skeletal muscle force developed at low frequencies of stimulation (Howell et al 1981, Jones et al 1982), increased force has not been identified at high frequencies (Jones et al 1982).

Nevertheless, *in vivo* studies report increased inspiratory muscle strength with aminophylline in animals (Howell et al 1985, 1986) and in patients with COPD (Murciano et al 1984, 1989). In the case of the animal studies, inotropic effects on muscle strength have been seen only at methylxanthine levels above the therapeutic range.

In order to determine if therapeutic levels of aminophylline result in increased respiratory muscle strength, we initially studied normal subjects, with the aim of later studying patients with COPD. In order to compare the effects on the respiratory muscles with those on skeletal muscle in general, we also studied the quadriceps femoris muscle.

AIMS

The primary aim was to determine the effect of aminophylline at therapeutic concentrations on the strength of the global respiratory muscles and specifically the diaphragm, and on the strength of the quadriceps femoris.

In addition, the endurance of the quadriceps was

investigated, in order to assess a possible effect on fatigue prevention.

SUBJECTS

Five healthy non-smoking subjects (four males) were recruited from the Cardiothoracic Institute of the Brompton Hospital. All were familiar with the techniques involved and two had performed the manoeuvres on several previous occasions. All gave their informed consent.

METHODS

Maximal static inspiratory mouth pressures after a full expiration (PImax), maximal static expiratory mouth pressures (PEmax), maximal sniffs, and maximal voluntary contractions of the quadriceps femoris (MVC) were performed as previously described in Chapter 2, Sections 1 and 2. A series of manoeuvres for each parameter were recorded. Three technically satisfactory recordings of both mouth pressure measurements were made in the morning and in the afternoon of each study day. Ten maximal sniffs were also measured once on each day. Three maximal voluntary contractions for each leg were recorded once each day, one leg in the morning and the other in the afternoon, the order being kept the same throughout the study.

QUADRICEPS ENDURANCE

This measurement was based on a method of fatigue-production described by Wiles and co-workers (1983). The aim was to monitor the time taken for subjects to fail to reach a target force after repeatedly achieving it.

A target force of 60% of the maximal voluntary contraction for each leg was set. The subject was asked to contract the quadriceps sharply to the target, holding the force for 1.5 seconds, and then relaxing the leg for 1.5 seconds. Fatigue was defined as failure to maintain 90% of the target for two consecutive contractions. The time to fatigue was termed quadriceps endurance and was measured once for each leg on each study day.

Placebo and slow-release aminophylline tablets (Phyllocontin Continuus 225mg) were supplied by Napp Laboratories.

PROTOCOL

This was a double-blind placebo-controlled cross-over study, with a protocol as described in Chapter 2, Section 4, Pages 174 & 175, Figure 2.12. An acclimatisation period comprising several measurement sessions spaced over 10 days enabled all subjects to master the techniques of mouth pressure and quadriceps strength and endurance measurements. It also ensured that the learning effect previously noted for serial mouth pressure measurement in normals was achieved before formal studies began (Brophy et al 1985a).

Two control days followed, during which the measurement protocol was performed. Maximal sniffs were measured only on the second day. At the end of each 5-7-day treatment period, the full schedule of measurements were carried out 3-6 hours after drug ingestion. Blood samples for theophylline assay were taken on both aminophylline and placebo study days between 5-8

hours after dosing.

STATISTICAL ANALYSIS

Mean maxima for each measurement session were compared using two-way analysis of variance to determine treatment effects. Mean and standard error of the mean [SEM] are presented for group results.

RESULTS

The subjects were aged from 28 to 32 years (mean 29 years).

Coefficients of variation for acclimatisation period measurements are given in Chapter 2, Section 3, Pages 163 and 172, and Table 2.8. Both mouth pressures and maximal voluntary quadriceps contractions were reproducible within and between days, allowing a single maximum value to be used for each treatment period, and ensuring between day comparisons were valid. This was not the case for the quadriceps endurance measurements (Chapter 2, Section 3, Page 172), which consequently will not be presented for the present study.

Sniff Pdi was greater on aminophylline than on placebo ($P<0.05$, on placebo, 137cmsH_2O; on aminophylline, 142cmsH_2O) (Table 6.1). There were no other statistically significant treatment differences. Individual values are presented in Figures 6.1-6.4.

Mean maximum mouth pressures and quadriceps voluntary contractions for the two control days were also compared (Table 6.2). There were no statistically significant differences in values between the control days.

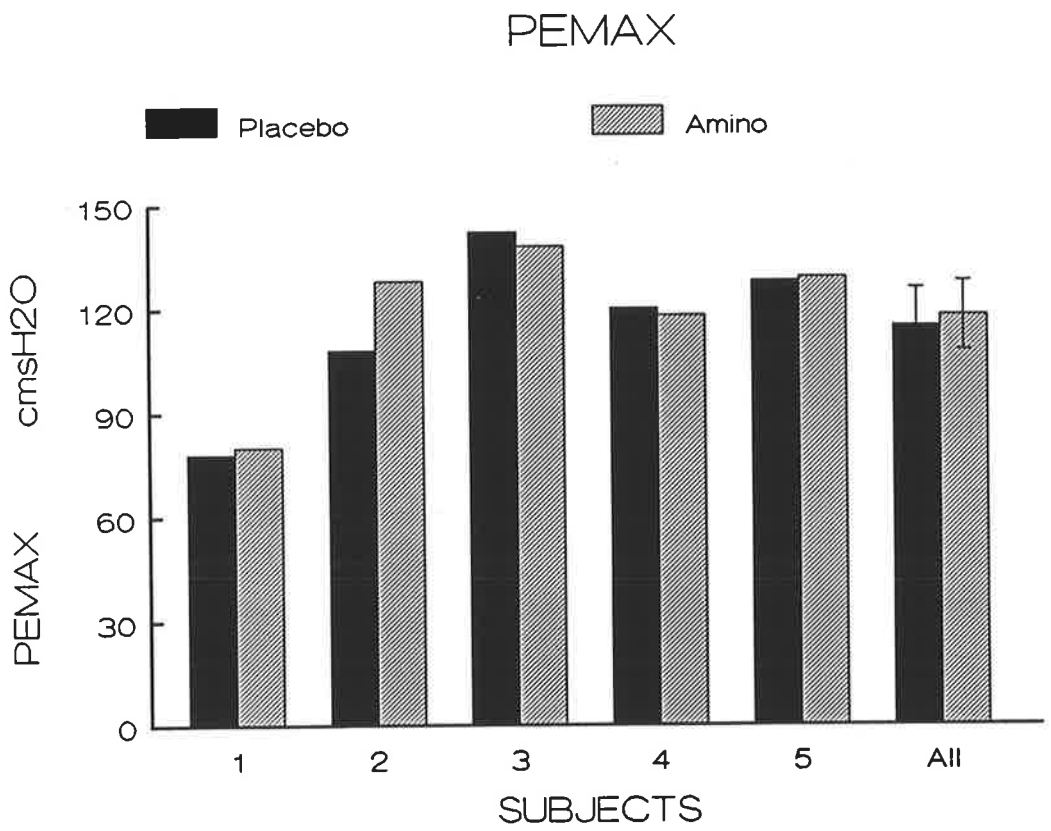
TABLE 6.1

MAXIMUM VALUES

	PLACEBO	AMINO	DIFF
PARAMETER	MEAN [SEM]		%
PEMAX (cmsH2O)	115 [11]	118 [10]	2.8
PIMAX (cmsH2O)	101 [12]	106 [15]	4.8
SNIFF PDI (cmsH2O)	137 [12]	142 [13]	4.1*
LT MVC (kgs)	47 [4]	48 [4]	1.5
RT MVC (kgs)	48 [5]	50 [4]	2.9

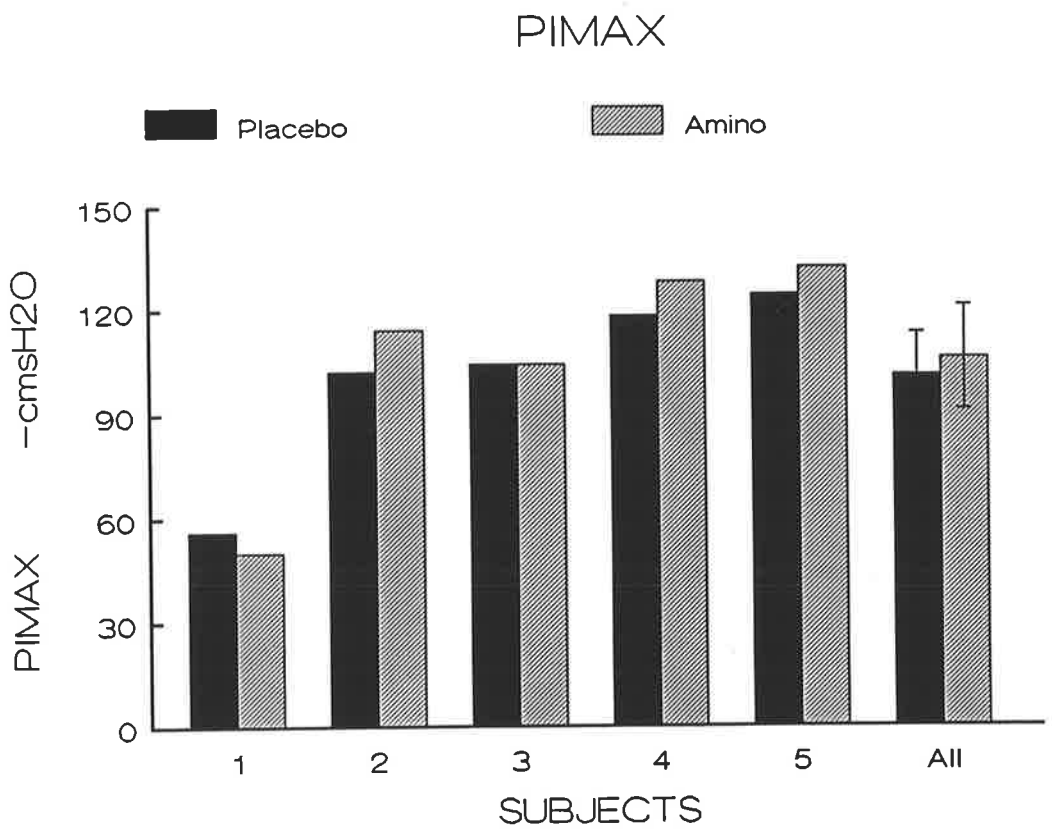
Mean maxima for maximal static expiratory and inspiratory mouth pressures (PEmax & PImax), maximal sniff transdiaphragmatic pressures (Sniff Pdi) and left and right maximal voluntary quadriceps contraction (Lt & Rt MVC) for the group, on placebo and aminophylline study days. The difference between maximal sniffs on aminophylline and on placebo was statistically significant (*P<0.05).

FIGURE 6.1



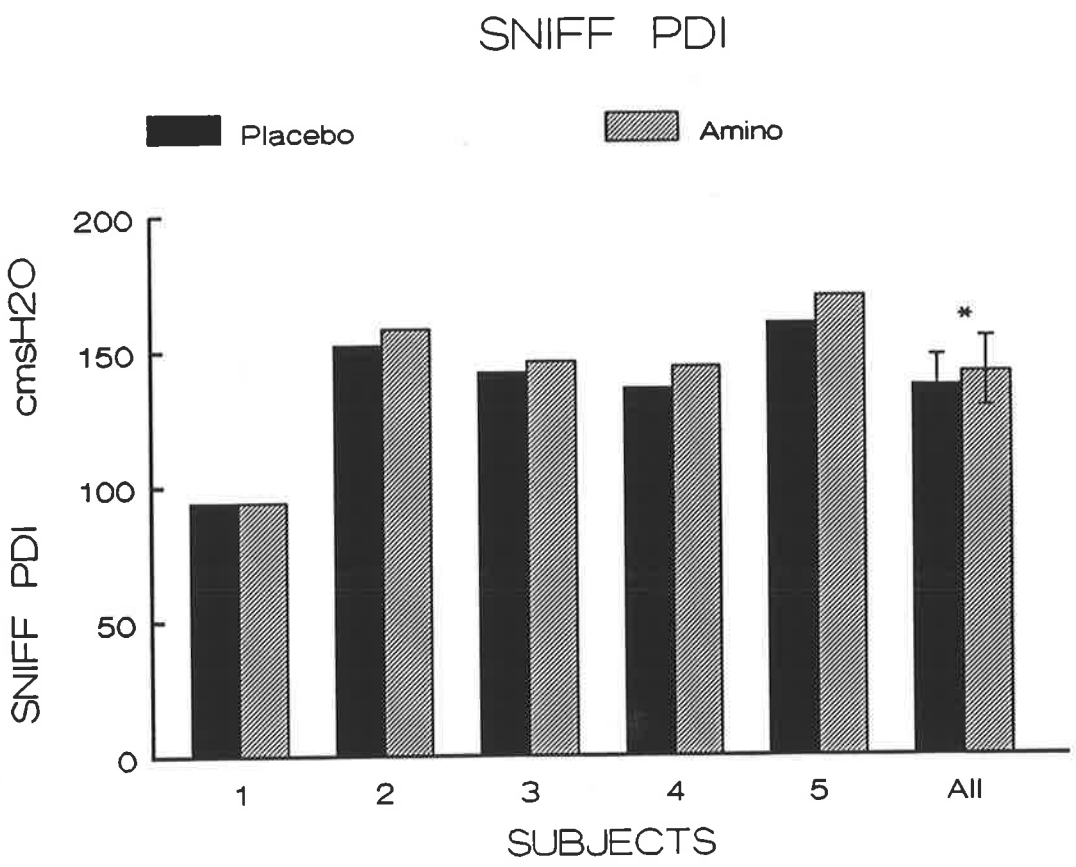
Maximal static expiratory mouth (PEmax) for the placebo and aminophylline treatment days and for each subject.

FIGURE 6.2



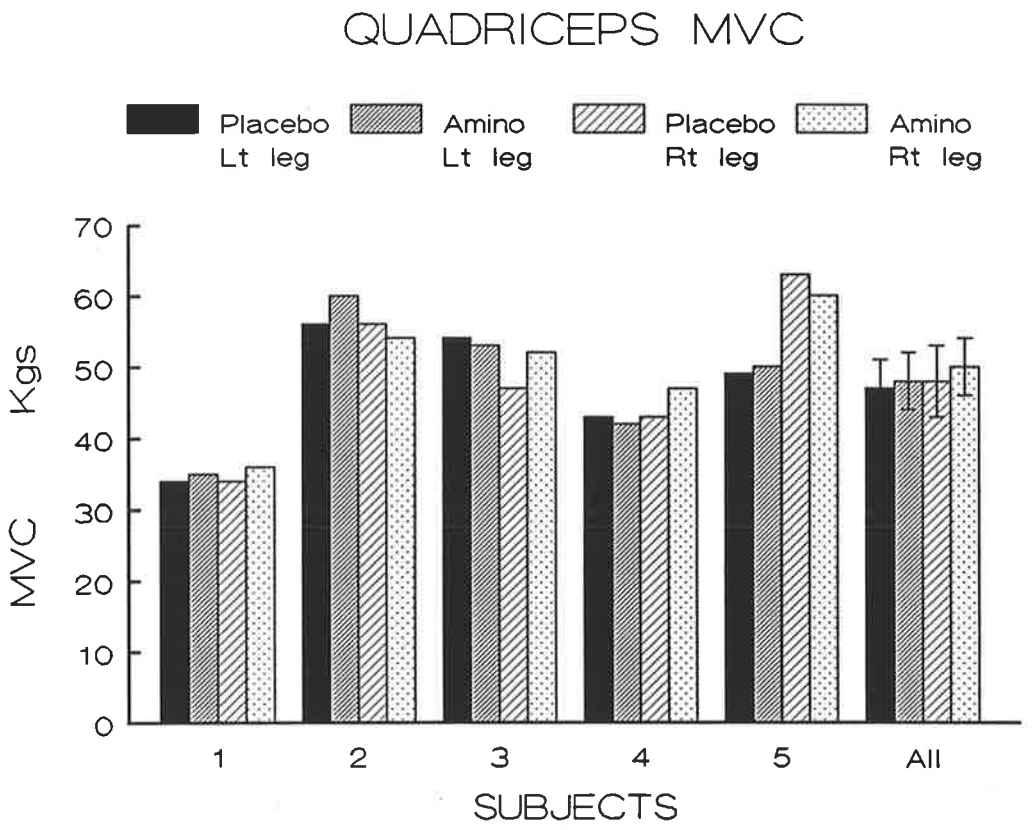
Maximal static inspiratory mouth (PImax) for the placebo and aminophylline treatment days and for each subject.

FIGURE 6.3



Maximal sniff transdiaphragmatic pressures (Sniff Pdi) for the placebo and aminophylline treatment days and for each subject. The group mean maxima were statistically significantly greater on aminophylline (*P<0.05).

FIGURE 6.4



Maximal voluntary quadriceps contractions (MVC) for the left and right legs for the placebo and aminophylline treatment days and for each subject.

TABLE 6.2

MAXIMUM VALUES

	DAY 1	DAY 2	DIFF
PARAMETER	MEAN [SEM]		%
PEMAX (cmsH2O)	114 [10]	111 [13]	-2.6
PIMAX (cmsH2O)	92 [10]	95 [13]	3.3
LT MVC (kgs)	45 [4]	45 [4]	0.0
RT MVC (kgs)	46 [4]	47 [4]	2.2

Mean maximum [SEM] values for maximal static expiratory mouth pressures (PEmax), maximal static inspiratory mouth pressures (PImax), left and right maximal voluntary quadriceps contractions (Lt & Rt MVC).

All but one subject (Subject 3; 8.4mg/l) had serum theophylline levels within the therapeutic range. The mean ±SD was 14.6mg/l ±6.3, and the range was 8.4-25.0mg/l.

One subject (No. 2) had an episode of palpitations lasting several hours during the first two days of dosing, but prior to allocation of test treatment. The theophylline concentration was checked, and was within the therapeutic range. The dose was stopped and the drug reintroduced at a lower dose, and increased again very slowly. There was no repetition of the event, and no further serious side effects were reported.

DISCUSSION

Sniff transdiaphragmatic pressure was 4.1% greater on aminophylline than on placebo. This suggests that a genuine improvement in inspiratory muscle strength was achieved with the drug. No other statistically significant changes occurred. These findings may be related to differences in the type of manoeuvre: the sniff is a short, sharp inhalation, whereas the performance of mouth pressures and quadriceps femoris MVCs requires maintenance of force for a short period. In addition, the sniff is a spontaneous manoeuvre familiar to subjects, and does not need to be learnt, in contrast to the other measurements which are initially unfamiliar to subjcts and require training. For these reasons, the sniff may be less effort-dependent than the other manoeuvres, and a possible effect on muscle contractility may be, therefore, more likely to be identified.

Although none of the mean differences in other parameters

were statistically significant they all favoured the active drug and were of the same magnitude (Table 6.1: 1.5%-4.8%). Changes in sniff transdiaphragmatic pressure and maximal static inspiratory mouth pressure were in the same direction and of similar magnitude for three of the five subjects (Nos. 2, 4 & 5) and for the group overall (Figures 6.2 & 6.3).

The increases seen with aminophylline were similar for both respiratory muscle and quadriceps strength measurements, suggesting that aminophylline affects the two types of muscle, in normal subjects, to the same extent. This is consistent with in vitro work (Jones et al 1982).

The small benefit in maximal sniff was considerably less than the effect on voluntary muscle contraction reported by some workers (Aubier et al 1981a, Murciano et al 1984, Supinski et al 1984a). Others have failed to find increases in skeletal muscle force in normal subjects (Efthimiou et al 1986, Lewis et al 1986) or in patients with COPD (Cooper et al 1985b, Kongragunta et al 1988).

Aubier et al (1981a) found a 15% increase in voluntary inspiratory muscle contractility, while Supinski et al (1984a) reported an increase of 16%. These two groups studied the acute effects of intravenous aminophylline and a single oral dose of theophylline respectively, whilst the present study was the first using more chronic administration of oral aminophylline. It is possible that acute administration produces a greater effect than chronic treatment, during which the action might subside.

Other studies of short duration in normal subjects support

the validity of the small changes reported in this study. Efthimiou and co-workers (1986) administered aminophylline intravenously to normal subjects on a single day and measured the effect on fatiguing work, strength and maximal relaxation rate of the sternomastoid. No action was found on the strength or on the susceptibility or extent of fatigue. Similar results were reported by Lewis and colleagues (1986).

These two studies differed from the present work only in the length of treatment, and in the induction of fatigue, but were similar in design to the studies by Aubier and co-workers (1981a), and Supinski and colleagues (1984a).

It is possible that the sternomastoid differs from other respiratory muscles in its sensitivity to methylxanthines. This seems unlikely, however. The muscle has been found to behave similarly to respiratory muscle with respect to the development of low frequency fatigue (Moxham et al 1980), while patients with COPD can develop fatigue of the sternomastoids following maximal voluntary ventilation and exercise (Wilson et al 1984b).

The results of the current work favour those of studies in patients with COPD showing minimal or no effect.

Cooper and co-workers (1985b) were unable to identify a benefit in patients with COPD following a two-week period on theophylline at therapeutic blood levels. No change in maximal static mouth pressures or lung volumes was noted.

Kongragunta and colleagues (1988) studied patients with mild COPD after three days on aminophylline and placebo. In

four of the eight patients studied, inspiratory muscle strength increased, while in the other four there was either no change or a decrease in strength with aminophylline. The drug did not prevent fatigue occurrence.

In contrast, Murciano and colleagues (1984, 1989), who investigated the effect of chronically administered methylxanthines on inspiratory muscle strength in COPD patients, reported substantial increases at therapeutic blood levels. Their results might be due, in part, to improvements in lung function and possibly reduction in lung volumes, which could have improved the diaphragmatic length-tension relationship, thereby increasing diaphragmatic contractility.

The magnitude of effect seen in the current study is also consistent with that seen in animal studies (Sigrist et al 1982) at blood levels corresponding to therapeutic doses in man. <u>In vitro</u> studies have consistently shown the effect of aminophylline to be dose related (Jones et al 1982, Shee et al 1983), and extrapolation from those data indicates that at therapeutic drug levels, the magnitude of a positive inotropic effect is likely to be below 10%.

The present study identifies such small changes and supports these predictions.

STUDY CRITIQUE

Motivation and cooperation are vital to the satisfactory performance of measurements dependant on volition. The subjects were highly motivated, as shown by the low coefficients of variation for repeated measurements. Subjects performed maximal

static mouth pressures and maximal voluntary quadriceps contractions (MVC) several times before starting the formal study. Mean coefficients of variation for PEmax and PImax measurements during this acclimatisation period were 7.6% and 7.4% respectively and for MVCs was 3.8%. (Chapter 2, Section 3, Pages 163 & 172). In addition, comparison of the two control day values, performed after the acclimatisation period, revealed no significant differences.

Maximal sniffs have a coefficient of variation of 5% on different days in normal subjects (Miller et al 1985), and do not have a large learning effect.

Despite the low variability in daily maxima (Chapter 2, Section 3, Pages 163 & 172, Table 2.8), the difference between treatment days in mouth pressures and quadriceps MVC were not detectable. A greater cohort would improve the chances of detecting a statistically significant difference.

The design and protocol used here produced distinct advantages as discussed in Chapter 3, Study Critique, Page 206.

Chest wall configuration was not controlled during mouth pressure and sniff manoeuvres. As described in Chapter 2, Section 1, PEmax and PImax vary little near total lung capacity or residual volume respectively (Agostoni & Rahn 1960). Vital capacity, which was performed immediately before each static manoeuvre, did not appreciably alter for any individual; hence, slight changes in the lung volume at which mouth pressure measurements were made would have had little effect on results.

Sniffs were performed from resting end-expiration. From calculations based on regression equations relating lung volume

changes to sniff transdiaphragmatic pressure (Wanke et al 1990), a mean volume change in functional residual capacity of approximately -300mls (ie. towards residual volume) would be required to produce the increases in sniff transdiaphragmatic pressure reported here.

Although total lung capacity and functional residual capacity were not measured in these subjects, vital capacity measurements were performed on the spirometer on each study day. Differences in vital capacity between the placebo and aminophylline days amounted to approximately 100mls. Estenne et al (1980), reported that there were no significant changes in lung mechanics, specifically in vital capacity, functional residual capacity, total lung capacity or expiratory reserve volume, in normal subjects given up to 8mg/kg of aminophylline. The authors found mean greatest non-significant differences in vital capacity and functional residual capacity of 80mls and 120mls respectively. On the basis of these studies it appears unlikely that the positions of end-expiration in the normal subjects reported here fell sufficiently to explain the changes in sniff transdiaphragmatic pressure.

CHAPTER 7

STUDY 5

THE EFFECT OF THEOPHYLLINE ON RESPIRATORY AND QUADRICEPS FEMORIS MUSCLE STRENGTH, PULMONARY FUNCTION AND BREATHLESSNESS IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

INTRODUCTION

There have been eleven studies of the effect of dimethylxanthines on inspiratory muscle contractility, ventilatory endurance or exercise performance in patients with chronic obstructive pulmonary disease (COPD). Four found improvements in inspiratory muscle strength, resistance to diaphragmatic fatigue and/or exercise capacity (Murciano et al 1984, 1989, Nietrzeba et al 1984, Mahler et al 1985). Two investigations reported small increases in ventilatory capacity (Belman et al 1985) or walking distance (Davidson et al 1984), but the authors concluded that this change was not clinically relevant. Five studies identified no improvement in diaphragmatic contractility, diaphragmatic fatigue or exercise tolerance (Eaton et al 1982, Evans 1984, Cooper et al 1985b, Foxworth et al 1988, Kongragunta et al 1988).

At the time the work for this thesis began, there were no reports of the effects of chronic theophylline on inspiratory muscle contractility, exercise tolerance and lung function measurements in the same patients. We wished to perform such a study in order to interrelate the multiple effects of theophylline in chronic obstructive pulmonary disease.

AIMS

The primary aim was to determine the effects of theophylline on respiratory muscle and quadriceps femoris strength in patients with COPD ie. chronic airways obstruction and hyperinflation.

Secondary aims were:

1. to identify whether respiratory muscle weakness occurs in patients with severe irreversible airways disease.

2. to determine in these patients the strength of the quadriceps femoris, the function of which is unaffected directly by severe lung pathology.

3. to compare the effect of theophylline on subjective and objective indices of COPD, and assess their relationship to a change in respiratory muscle function.

PATIENTS

All patients were recruited from the out-patient population of the Brompton Hospital. The entry criteria were:-

1. a clinical diagnosis of COPD with moderately severe airways obstruction.

2. irreversibility of airways obstruction defined as a failure to improve FEV_1 by 20% or more following inhalation of 400 mg of salbutamol aerosol.

3. willingness to devote the time and effort to the study.

4. informed verbal consent.

METHODS

The methods and equipment used for the performance of maximal static mouth pressures, quadriceps femoris strength, pulmonary function, and transdiaphragmatic pressures are as described in Chapter 2, Sections 1 and 2. The methods and equipment used for the performance of peak expiratory flow rate, scoring of general well-being and breathlessness by visual analogue scale, and six-minute walk are described below.

VISUAL ANALOGUE SCALES

Visual analogue scales were used as scoring systems for general well-being and for breathlessness (Figure 7.1). The scales comprised a straight line, 100mm in length, with statements of reference at each end (Aitken 1962). Patients were asked to make a single firm stroke across the line to indicate their average position on the scale for the preceding week.

FIGURE 7.1

GENERAL STATE

Couldn't feel worse generally — Couldn't feel better generally

STATE OF BREATHLESSNESS

No breathlessness — Extreme breathlessness

The two visual analogue scales used for scoring general well-being and breathlessness. Patients made a single firm stroke across the line. The distance from the left-side to the written stroke was measured in millimetres and recorded. The values were arcsine transformed prior to analysis.

PEAK EXPIRATORY FLOW RATE (PEFR)

Peak expiratory flow rate (PEFR) was performed at each weekly visit with a Wright mini-peakflow meter. Patients were

required to breath to total lung capacity and then to perform a sharp, maximal expiration through the meter. The best of two or more technically satisfactory procedures was noted.

SIX-MINUTE WALK

The six-minute walking test were performed on the flat and inside in a warm environment, along a well-lit unobstructed 50 metre-long corridor (Butland et al 1982). Subjects were told to walk as fast and for as long as they were able, and that they would be asked to stop after six minutes. If they needed to stop because of breathlessness before this they could do so and they were asked to resume the walk as soon as possible. The time for which they rested was included in the six-minute count.

PROTOCOL

The protocol and design were as described for chronic studies in Chapter 2, Section 4, Pages 174 & 175, Figure 2.12. Patients were assessed on several occasions during a pre-trial acclimatisation period to ensure that they were able to perform the muscle strength measurements, and to gauge their commitment to the study. The results of repeatability measurements are presented in Chapter 2, Section 3, Page 169, Table 2.8 & Page 173, Table 2.9.

At the end of this period, the patients were given Uniphyllin SR (theophylline 225mg tablets, Napp Laboratories) unblind. In order to to limit toxic side effects, the initial dose was low, and was increased gradually over several days. Patients already on a dimethylxanthine stopped that treatment

and had theophylline levels checked before starting Uniphylline SR. Blood samples were taken for theophylline assay, in order to determine the dose giving a therapeutic level. Patients were then allocated, randomly and double-blind, to treatment with either placebo or theophylline at this dose. The same dose of the alternative treatment was used in the second treatment period, unless side effects during the intervening unblind period necessitated further dose-titration.

Every week for each six-week treatment period, patients attended the laboratory for the performance of the test procedures. Trial treatment was stopped at the end of the study, and those patients previously on a dimethylxanthine were represcribed that treatment.

All patients had 24 hour-a-day access to medical advice by phone if the need arose. They were instructed to contact the respiratory muscle laboratory immediately symptoms of an exacerbation occurred, so that the appropriate treatment could be initiated. Persistent symptoms warranting the introduction of new concomitant medication for more than five days resulted in the withdrawal of the patient from the trial.

All medications except pre-trial dimethylxanthines were continued. Oral steroids were allowed as long as the dose was not changed during the study.

Coffee and tea intake was limited to two cups of either within the six hours preceding testing. Soft drinks containing methylxanthines were not allowed prior to attendance at the laboratory.

MEASUREMENTS

At each visit the following measurements were made:

1. Maximal static mouth pressure from total lung capacity (PEmax), maximal static inspiratory mouth pressure from residual volume (PImaxRV) and from resting end-expiration (PImaxFRC).

2. Right and left quadriceps femoris maximal voluntary contractions (MVCs).

3. Peak expiratory flow rate (PEFR).

The best three of a series of manoeuvres were recorded.

4. Breathlessness and general well-being scores, on visual analogue scales.

In addition, at the end of the two treatment periods a further set of measurements were performed. These comprised:-

1. Seated and supine or semi-recumbant vital capacity.

2. Pulmonary function.

3. Six-minute walk.

4. Venous blood sampling for theophylline assay, approximately 12 hours after dosing.

In patients who consented to have balloon catheter measurements, transdiaphragmatic pressures were also recorded during the following:

1. Tidal breathing and vital capacity manoeuvres.

2. Maximal sniffs. The best 10 maximal sniffs were recorded.

3. Maximal inspiratory efforts. The best three were recorded.

4. Unilateral and bilateral twitches produced by

transcutaneous phrenic nerve stimulation. A minimum of five maximum twitches were measured during right, left and bilateral phrenic nerve stimulation.

All measurements were performed at the same time of day at all visits.

STATISTICAL ANALYSIS

Data for the last three weeks of each treatment period only were considered, as by this time patients were stabilised on the assigned therapy. In addition, by waiting for three weeks, the effects of long-term rather than acute treatment were studied. Comparison of data was by two way analysis of variance. Visual analogue scale data were first handled by arcsine transformation, in order to produce normal data distribution. The mouth pressure data for two patient sub-groups were also separately analysed. The first sub-group comprised the five patients who had transdiaphragmatic pressure measurements, and the second consisted of those who did not have these recordings.

The primary end-points were mouth pressure measurements.

RESULTS

Thirteen patients (three females) were entered to the pre-trial period; only 10 (two females) felt able to continue into the treatment phase of the study. Reasons for failure to continue were in all cases related to inconvenience or inability to make the required time committment. No patient was withdrawn or dropped out during treatment.

The age range of patients entered to the treatment phase of the study was 43 - 73 years, with a mean of 60 years (Table 7.1). All were non-smokers, although all but one patient (No. 4) had smoked in the past, the majority for many years.

PREADMISSION PULMONARY FUNCTION (TABLES 7.1, 7.2 & 7.3)

Preadmission pulmonary function was moderately to severely impaired (FEV_1:FVC ratio: 36.1%), with airflow rates markedly reduced (FEV_1: 28.8% of the predicted value) (Table 7.2). All but two patients had irreversible airflow obstruction, as defined in the entry criteria (Table 7.1) (Reversibility: No. 3 - 20%; No.10 - 23%). The patients were selected for inclusion on the basis of previous data which did fulfill this entry criterion. The subsequent preadmission tests showed that these two patients had greater reversibility, but they were included because the degree of reversibility was small, they fulfilled the other criteria, and because suitable patients willing to participate in this invasive study were very difficult to find.

Total lung capacity was 125.2% of the predicted value, and residual volume was 205.3% of the predicted value, indicating that patients had hyperinflation (Table 7.3). In keeping with this, functional residual capacity was elevated (5135mls). Five patients (Nos. 1, 5, 7, 8 & 9) had raised pCO_2 values, and all had lowered pO_2 values (Table 7.2). Overall, the patients were hypoxic, and on the borderline between normocapnia and hypercapnia, (pO_2: 7.7kilopascals [kpa], pCO_2: 5.6kpa). (Normal ranges, pO_2: 11.5-13.5kpa; pCO_2: 4-5.5kpa, Brompton Hospital).

TABLE 7.1

PATIENT DETAILS

PATIENT No	GENDER	AGE (yrs)	PRIMARY DIAGNOSIS	REVERSIBILITY (%)
1	M	65	COPD	17
2	M	52	EMPHYSEMA	10
3	M	73	COPD	20
4	M	43	EMPHYSEMA	1
5	M	53	COPD	18
6	M	55	COPD	-3
7	F	50	COPD	5
8	F	65	COPD	-3
9	M	70	COPD	-4
10	M	69	CHRONIC BRONCHITIS	23
MEAN [SD]		59.5 [10.1]		8.4 [10.5]

Details of the 10 patients entered to the study, prior to entering the acclimatisation phase. Age (mean & standard deviation [SD]), gender, primary diagnosis and percentage (%) reversibility (mean & SD) to 400 micrograms of inhaled salbutamol are presented.

TABLE 7.2

BASELINE PULMONARY FUNCTION

PATIENT No.	FEV1 (mls)	FVC (mls)	FEV1/FVC (%)	pO2 (kpa)	pCO2 (kpa)
	(% predicted)				
1	520 (24)	1720 (57)	30	7.0	6.3
2	1500 (44)	2850 (64)	53	9.8	4.9
3*	680 (29)	2140 (65)	32	8.0	4.4
4	790 (22)	3180 (70)	31	8.6	5.1
5	760 (28)	2020 (57)	38	8.3	6.4
6	790 (24)	2540 (58)	31	9.8	4.4
7	560 (25)	1740 (65)	32	4.7	8.3
8	630 (29)	1560 (51)	49	5.8	5.8
9*	540 (18)	2300 (45)	28	7.0	5.6
10	1010 (45)	2720 (88)	37	4.7	4.8
MEAN	778 (29)	2238 (69)	36.1	7.4	5.6

*Placebo data used for patients 3 & 9

Pulmonary function for patients entered to the treatment phase of the study. Mean forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) in millilitres (mls), the ratio of FEV_1 to FVC as a percentage, and arterial blood gas values for oxygen (pO_2) and carbon dioxide (pCO_2) in kilopascals (kpa) are presented for the ten patients and the group, prior to entering the study.

TABLE 7.3

BASELINE PULMONARY FUNCTION

PATIENT No.	TLC (mls)	RV (mls)	FRC (mls)	AWR (kpa/l/sec)
	(% predicted)			
1	4760 (100)	2760 (150)	3410	1.09
2	9440 (147	6290 (291)	7860	0.25
3*	6470 (125)	4220 (214)	5650	0.66
4	7710 (122)	4510 (226)	5780	1.17
5	5140 (100)	2990 (170)	4360	1.43
6	7760 (119)	5410 (239)	6210	0.48
7	4480 (108)	2780 (201)	3400	1.50
8	5150 (104)	3500 (184)	4350	1.21
9*	7530 (106)	5030 (185)	6830	0.69
10	5740 (116)	3140 (165)	3770	0.72
MEAN	6390 (125)	4045 (205)	5135	0.92

*Placebo data used for patients 3 & 9

Further baseline pulmonary function values for the ten patients, prior to entering the study. Individual and group mean total lung capacity (TLC), residual volume (RV) and functional residual capacity (FRC) in millilitres (mls), and airways resistance in kilopascals/litre/second (kpa/l/sec) are presented. The % predicted values of TLC and RV are also given.

TREATMENT PERIOD STUDY PARAMETERS

Maximal Static Mouth Pressures

Mean maximal static inspiratory mouth pressures at residual volume (PImaxRV) and end-expiration (PImaxFRC) over the final three weeks of treatment were statistically significantly greater on theophylline than on placebo. The mean difference in PImaxRV was 11.4% ($P<0.05$, 64.1cmsH_2O on placebo, 70.2cmsH_2O on theophylline), and in PImaxFRC was 18% ($P<0.01$, 51.3cmsH_2O on placebo, 58.1cmsH_2O on theophylline) (Figure 7.2). There were no differences in maximal static expiratory mouth pressures (PEmax: 104.6cmsH_2O on placebo, 107.3cmsH_2O on theophylline).

Maximal Voluntary Quadriceps Contractions (MVC)

There were no improvements in quadriceps muscle strength: (MVC right leg: 29.6kgs on placebo, 30.1kgs on theophylline; left leg: 27.5kgs on placebo, 28.4kg on theophylline) (Figure 7.2).

Pulmonary Function

There were no changes in residual volume (RV) or functional residual capacity (FRC) (RV: 4334mls on placebo, 4424mls on theophylline; FRC: 5486mls on placebo, 5406mls on theophylline), in airway calibre (FEV_1: 693mls on placebo, 686mls on theophylline; PEFR: 173.3l/min on placebo, 191.8l/min on theophylline) (Figure 7.3 & Table 7.4), or in airway resistance (AWR: 0.75kpa/l/sec on placebo, 0.76kpa/l/sec on theophylline) (Table 7.4).

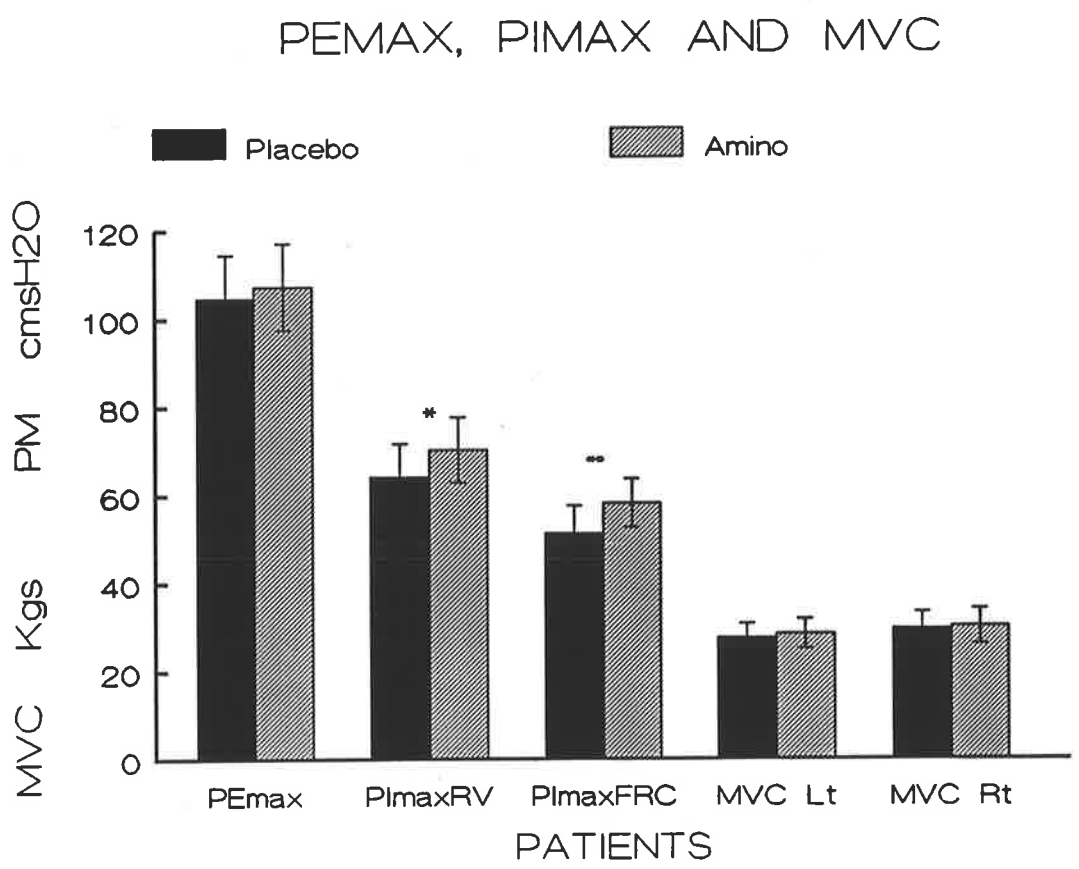
Visual Analogue Scales

Subjective indices of well-being and breathlessness were not altered with active treatment (Table 7.4).

Six-Minute Walk

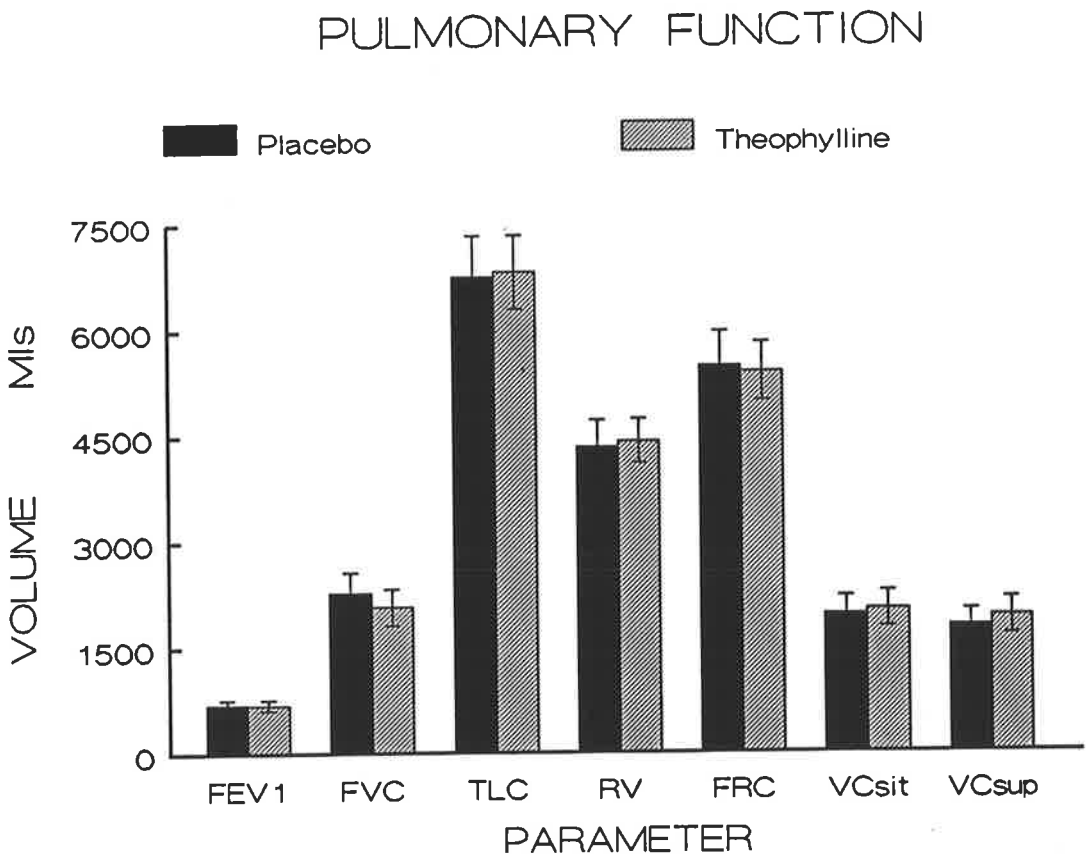
Walking distance was not affected by theophylline (six-minute walk, mean: 356 metres on placebo, 382 metres on theophylline) (Table 7.4).

FIGURE 7.2



Mean [SEM] maximal static expiratory mouth pressures (PEmax), maximal static inspiratory mouth pressures at residual volume and functional residual capacity (PImaxRV & PImaxFRC) in $cmsH_2O$, and maximal voluntary quadriceps contractions (MVC) for the right (Rt) and left (Lt) legs in kilograms (kgs) for the placebo and theophylline treatment periods. Values for PImaxRV & PImaxFRC were statistically significant greater on theophylline. *P<0.05. **P<0.01.

FIGURE 7.3



Mean [SEM] pulmonary function for placebo and theophylline periods. Data on forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), total lung capacity (TLC), residual volume (RV) and functional residual capacity (FRC), and vital capacity (VC) sitting (sit) and supine (sup) are presented. Units are millilitres (mls). There were no statistically significant treatment differences.

***n=8**

TABLE 7.4

SIX-MINUTE WALK, AIRWAYS RESISTANCE & PEAK FLOW RATE

	PLACEBO [SEM]	THEOPHYLLINE [SEM]
	(n=10)	
6MIN WALK (m)	356 [59]	382 [53]
AWR (kpa/l/sec)*	0.75 [0.11]	0.76 [0.15]
PEFR (l/min)	173.3 [14.8]	191.8 [14.5]

Six-minute walking distance in metres (m), airways resistance (AWR) in kilopascals/litre/second (kpa/l/sec), and peak expiratory flow rate (PEFR) in litres per minute (l/min) for the group. Six-minute walk and airways resistance was measured at the end of each 6-week treatment period, and PEFR was measured at each weekly visit.

***n=9.**

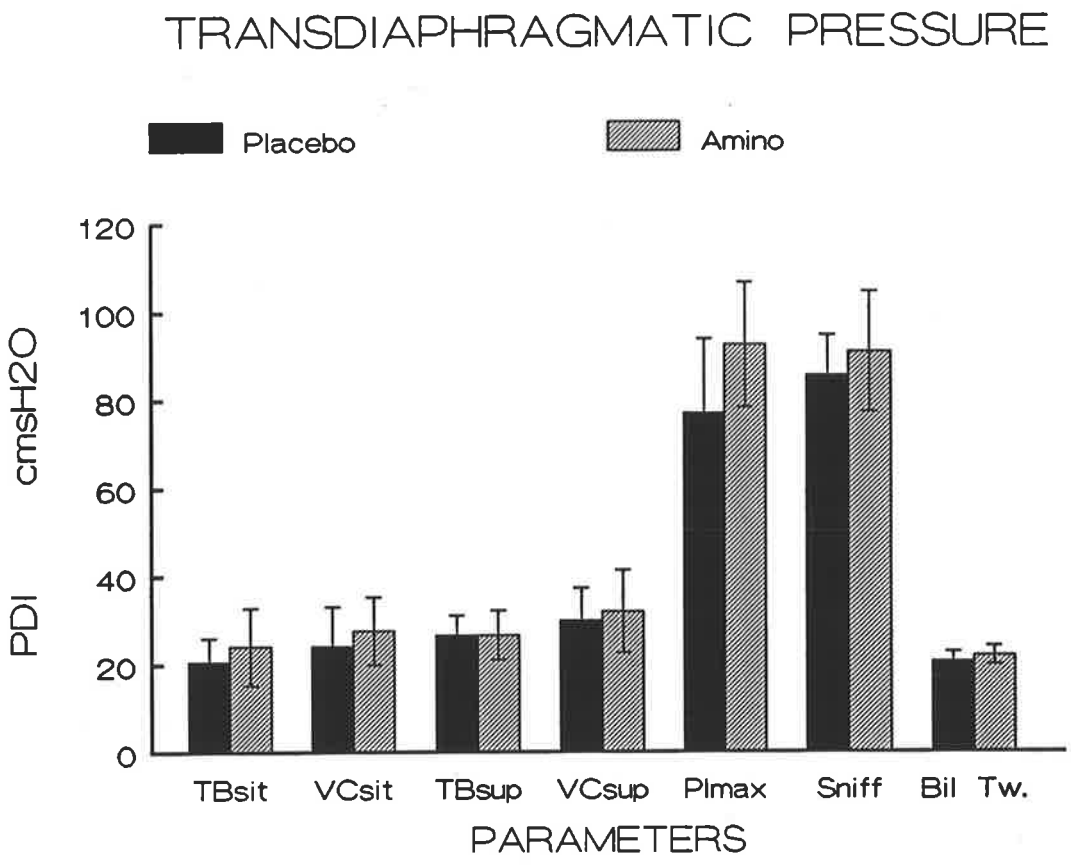
Transdiaphragmatic Pressures

Five of the 10 patients (Nos. 3, 4, 5, 9 & 10) had transdiaphragmatic pressure measurements (Figure 7.4). Bilateral phrenic nerve stimulation was performed in only four of these five patients; in the fifth (No. 9) bilateral twitches could not be elicited. There were no statistically significant differences between placebo and theophylline values (PdiPImaxRV: 77.0cmsH_2O on placebo, 92.5cmsH_2O on theophylline; sniff Pdi: 85.5cmsH_2O on placebo, 91.0cmsH_2O on theophylline; twitch Pdi: 20.5cmsH_2O on placebo, 21.9cmsH_2O on theophylline).

Maximal Static Inspiratory Mouth Pressures for Sub-groups

The maximal inspiratory mouth pressures for these five patients and the other five who did not have transdiaphragmatic pressure measurements were analysed separately. PImaxFRC values for the second subgroup were greater on theophylline than on placebo. No other treatment differences for PImaxRV or PImaxFRC for the subgroups were statistically significant. Values for patients who had Pdi measurements were PImaxRV: 68.9cmsH_2O on placebo, 76.0cmsH_2O on theophylline, p=0.060; PImaxFRC: 57.6cmsH_2O on placebo, 64.5cmsH_2O on theophylline, p=0.078. Values for patients who did not were PImaxRV: 59.2cmsH_2O on placebo, 64.4cmsH_2O on theophylline, p=0.238; PImaxFRC: 44.9cmsH_2O on placebo, 51.6cmsH_2O on theophylline, p=0.017.

FIGURE 7.4



Mean [SEM] transdiaphragmatic pressure (Pdi) measurements in cmsH_2O during tidal breathing and vital capacity manoeuvres, maximal inspiratory efforts (PdiPImax), maximal sniffs, and bilateral phrenic nerve stimulation (twitch Pdi). Phrenic nerve stimulation was achieved in only four patients. There were no statistically significant treatment differences.

Individual Results

Seven patients had statistically significant increases in one or both maximal static inspiratory mouth pressures with theophylline (Table 7.5). Three patients showed increased peak expiratory flow rates on theophylline; two of these individuals also had improved maximal static inspiratory mouth pressures from functional residual capacity.

TABLE 7.5

PATIENT NO.	PARAMETER
1	PEmax, PImaxFRC, PEFR.
2	PEFR, MVC Lt leg, VAS general.
3	PImaxRV.
4	VAS breathlessness.
5	PImaxFRC.
6	PImaxRV, PImaxFRC.
7	PImaxFRC.
8	PEmax, PImaxRV, PImaxFRC, MVC Lt leg, VAS breathlessness.
9	VAS general.
10	PImaxFRC, PEFR.

TABLE 7.5

The parameters for which individual patients showed statistically significant treatment differences.

Theophylline Levels

All but two patients (No. 7: 9.4mg/l & No.8: 8.9mg/l) had serum theophylline levels values within the therapeutic range; the mean ±SD was 12.1mg/l ±2.9, and the range was 8.9-15.0mg/l.

No side effects were reported during the double-blind treatment periods.

DISCUSSION

The patient group had moderately severe airways obstruction with poor reversibility, hyperinflation and hypoxia. The maximal inspiratory mouth pressures were below those predicted (Wilson et al 1984a). Thus, the inspiratory muscle function of these patients should have been susceptible to improvement.

There was an increase in global inspiratory muscle strength measured by static inspiratory mouth pressures recorded at residual volume and at the end of a relaxed expiration. Maximal static expiratory mouth pressure values showed no improvement with theophylline and this, together with unchanged quadriceps strength, implies that theophylline had no effect on skeletal muscle in general. Either the effect on the inspiratory muscles was related to the heavy work loads which these muscles bear in patients with airway obstruction, hypercapnia and hypoxia, or there was no genuine inotropic effect on skeletal muscle. In the latter case, the enhanced inspiratory muscle strength results may have been due to a combination of other actions; eg. a small increase in airway calibre together with a reduction in lung volume, and a consequent increase in inspiratory muscle length.

However, a change in lung volume was not evident from the overall values for residual volume and functional residual capacity. Similarly there were no substantial changes in airflow rates, or in airway resistance to explain the increased static mouth pressure measurements.

From the individual results, two patients with improvement in one or both parameters of global inspiratory muscle strength also showed an increase in peak expiratory flow rates with theophylline. This might suggest that the improvement in muscle strength in these patients was related to increased airflow. However, of the seven patients with statistically significant improvements in inspiratory muscle strength, five showed no concomitant change in peak expiratory flow rates.

The enhanced inspiratory mouth pressures can not be explained by the variability of the measurements, because all patients underwent a prolonged pre-trial acclimatisation phase, during which they repeatedly performed mouth pressure measurements. The coefficients of variability for measurements performed on separate days are presented in Chapter 2, Section 2, Pages 168 & 172, and are similar to those for pulmonary function measurements.

There was no subjective improvement either in general well-being or, specifically, in breathlessness, and no improvement in walking distance. Had the increase in inspiratory muscle strength been sufficient to allow increased exercise tolerance in these patients, the benefit might be expected to have been detected by the patient and to be translated into a subjective

improvement. However, the patients with increased inspiratory mouth pressures did not have a change in visual analogue scales. This suggests that the relatively small increments in maximal inspiratory mouth pressures were insufficient to be of clinical relevance.

There may be other reasons for the lack of change in visual analogue scales. Firstly, patients may require an improvement in airway obstruction before they notice relief of dyspnoea. Secondly, the scales may be insufficiently sensitive to detect a statistically significant difference. Thirdly, patient improvement may be less apparent at rest than during extreme exercise (Nietrzeba et al 1984).

There have been eleven other studies which have investigated the effect of dimethylxanthines in patients with COPD. Of these, seven included specific measurements of respiratory muscle function. Four failed to find any increase in inspiratory mouth pressures or transdiaphragmatic pressure (Davidson et al 1984, Cooper et al 1985b, Foxworth et al 1988, Kongragunta et al 1988), while three identified increased inspiratory muscle contractility (Murciano et al 1984, Nietrzeba et al 1984, Murciano et al 1989). Of the remaining four studies, three reported no increase in six minute or twelve minute walking distances (Eaton et al 1982, Evans 1984, Mahler et al 1985), although there was improvement in breathlessness scores in one (Mahler et al 1985), and one noted a small increase in ventilatory endurance, a change not considered by the authors to be clinically relevant (Belman et al 1985).

Two of the investigations (Murciano et al 1984, Nietrzeba et al 1984) did not include double-blind randomisation, the other studies were adequately controlled and double-blind. Six were short-term studies, while five investigated the action of theophylline when given for a week or more (Eaton et al 1982, Cooper et al 1984b, Mahler et al 1985, Murciano et al 1984, 1989).

Murciano and colleagues (1984) found increased inspiratory muscle contractility and endurance with chronic administration of aminophylline. This was a single-blind, parallel group study with 13 patients receiving theophylline, and six receiving placebo. Although individual details of lung volumes and changes in airways obstruction were not reported, the mean changes in functional residual capacity and forced vital capacity suggest that the improvements in transdiaphragmatic pressure and maximal inspiratory mouth pressures might have been brought about, in part, by a reduction in lung volume, and return of the flattened diaphragm to a more normal configuration, with improvement in the length-tension relationship of the diaphragm. In addition, patients did not undergo a learning period to accustom them to mouth pressure measurement. Some of the increase seen might, therefore, have been due to a learning effect.

In further work by this group, patients with COPD received eight weeks of treatment with placebo or theophylline, crossing over double-blind to the other agent after a two-week washout period (Murciano et al 1989). The authors used oesophageal

pressure as the marker of inspiratory muscle force. The ratio of oesophageal pressures during tidal breathing and maximal inspiratory efforts is thought to be an index of respiratory muscle reserve (Roussos & Macklem 1977). The ratio increased by 28.6%, 22% because of an increase in maximal oesophageal pressure.

Comparison of this work with other studies is most effectively made with oesophageal pressure during maximal efforts. This applies for three reasons. Firstly, most studies have used maximal efforts, whether Mueller or modified Mueller manoeuvres. Secondly, oesophageal pressure measured with a correctly positioned balloon catheter is the same as the pressure recorded at the mouth (Baydur et al 1982). Thirdly, from inspection of oesophageal pressure records, it is clear that pressure during tidal breathing can be very variable, while oesophageal pressure during a maximal inspiratory effort has good repeatability (Miller et al 1985). The benefit seen in the study by Murciano and colleagues (1989) can be considered to be 22% for purposes of comparison, a value similar to that found for PImaxFRC in the current study.

Nietrzeba et al (1984) studied patients with chronic airflow obstruction during exercise. Increased maximal static mouth pressures, maximal oxygen uptake, maximal work rate and exercise duration, and decreased breathlessness were noted. There were technical difficulties with the measurement of mouth pressures, however. The pressure meter did not measure pressures greater than $-60 cms H_2 0$, so that the increase with aminophylline was unknown. It is also possible that the device

was non-linear at the extreme of the measurement range, in which case measurements close to -60cmsH_2O might not have been accurate.

In keeping with the current work, others have failed to identify a clinically significant action of methylxanthines on exercise tolerance and breathlessness in COPD patients (Eaton et al 1982, Evans 1984, Cooper et al 1985b, Kongragunta et al 1988). The first two studies entailed measurement of six minute walks and breathlessness, but no respiratory muscle measurements (Eaton et al 1982, Evans 1984). The other three studies included mouth pressure or transdiaphragmatic pressure recorded during maximal inspiratory efforts (Cooper et al 1985b, Kongragunta et al 1988, Foxworth et al 1988). No change in respiratory muscle strength was seen in these studies.

While a significant increase in sustained ventilatory capacity was noted by Belman and colleagues (1985), the authors concluded that the level of improvement was not clinically relevant. Davidson and co-workers (1984) identified significant bronchodilatation in patients with COPD following two weeks of treatment with aminophylline. No change in maximal static mouth pressures or lung volumes was noted. A small increase in six minute walk occurred, but at the expense of an increase in breathlessness.

The chief difference between the negative studies, the present work, and that of Murciano and colleagues (1984, 1989), is the severity of disease, and particularly, the blood gas values. Patients in the negative studies had normal blood

gases, while those gaining improvements in inspiratory muscle contractility were hypoxic. Furthermore, patients with hypercapnia showed greater changes than those with normocapnia (Murciano et al 1989).

STUDY CRITIQUE

The current work studied the action of theophylline over a prolonged period, in order that a possible action of the drug on the respiratory muscles might have time to take effect. The primary measurement periods were the last three weeks of each treatment, so allowing a long period for carry-over effects to subside before the second measurement period.

In the large study by Murciano et al (1989), patients were studied for several weeks, with a crossover design as in the present work. However, in the former trial, patients were switched directly from one study treatment to the other, whereas in the present investigation, patients received the active drug again, unblind for several days.

The protocol used in our study had several advantages (Chapter 1, Section 6, Pages 174 & 175). Firstly, it allowed a further check on the correct dose should it prove necessary. Secondly, by gradually increasing the dose during the unblind period, there was no sudden increase in side effects in those patients changed from placebo to theophylline, and no sudden cessation of side effects in those going from active drug to placebo. The identity of both the preceding and succeeding study treatments were, therefore, effectively obscured.

A difference between this work and other published studies

is that mouth pressures were used as the primary parameter, rather than oesophageal or transdiaphragmatic pressures (Murciano et 1984, 1989, Kongragunta et al 1988, Foxworth 1988). All the inspiratory muscles are important during ventilation, and the function of inspiratory intercostals may be vital to a patient with bronchoconstriction and hyperinflation (Martin et al 1983). It is therefore relevant to consider all the muscles responsible for inspiration, rather than one alone, when determining the significance of a drug effect.

Only half the cohort in this investigation had transdiaphragmatic pressure recordings, and this lack of consistency resulted in loss of statistical power for these measurements. An inotropic effect on maximal static inspiratory mouth pressures was identified for the group as a whole. However, when analysed as two individual subgroups (five patients who did have transdiaphragmatic pressure recordings, and five patients who did not), a treatment difference was seen only for maximal inspiratory mouth pressures from functional residual capacity in the five patients who did not have transdiaphragmatic pressure measurements. This inconsistency between results for the subgroups and for the group as a whole suggests that the size of the subgroups was too small for differences to be detectable.

The mechanism of action is not clear from this study. Bronchodilatation or a change in lung volume do not appear to be responsible for the improvement in inspiratory mouth pressures. Increased inspiratory muscle contractility is one

possibility. Another is central nervous system stimulation with enhancement of neural output. The patients were hypoxic, and hypoxic ventilatory drive is increased by methylxanthines (Lakshminarayan et al 1978). Another possibility is that the hypoxia in these patients increased the sensitivity of both the ventilatory drive and inspiratory muscle contractility to methylxanthines.

CHAPTER 8

GENERAL DISCUSSION

INTRODUCTION

Despite intensive study, the effect of dimethylxanthines on the respiratory muscles remains controversial. It is agreed that the drug has an inotropic action *in vitro* on skeletal muscle strips, and specifically on respiratory muscle (Howell et al 1981, Jones et al 1982). However, there is no consensus on effects in man: whether there is an inotropic action on respiratory muscles at therapeutic concentrations, what is the magnitude of effect, and whether enhancement of maximal contraction as well as submaximal contraction occurs. The clinical relevance of any action has also not been determined.

The work in this thesis arose because of the clinical importance of the respiratory muscles and of respiratory muscle failure. The studies were planned to address the issues described above, and to investigate the clinical relevance of methylxanthines to patients with disorders of the respiratory muscles. The specialised investigations of respiratory muscle function were performed within a well-established research laboratory, the clinical through-put of which enabled detailed study of patients who might benefit from an increase in inspiratory muscle contractility.

FINDINGS

The first study (Chapter 3) confirms that low frequency fatigue can be produced in the quadriceps femoris of normal subjects. Low frequency fatigue, due to failure of electro-mechanical coupling in muscle, is thought to be the type of fatigue suffered by patients with COPD. This was the first

study to investigate the effect of chronic theophylline on the frequency-force relationship of the quadriceps femoris, and on fatigue resulting from stimulation of the muscle through its innervating nerves.

Pre-administration of oral theophylline did not prevent the occurrence of fatigue, as judged from the 20:100Hz ratio and the shape of the frequency-force curve. However, the drug did attenuate fatigue to a small extent; there was a less marked fall in force at 20Hz from the first to the third frequency-force curve with theophylline, of 2.2% before fatigue and 0.75% after fatigue.

The second study (Chapter 4) showed that acute administration of aminophylline had no effect on bilateral twitch tension in normal subjects. When twitches were selected, so that those during the control and aminophylline study periods were matched as closely as possible for lung volume, diaphragmatic configuration and electromyogram amplitude, the mean (non-significant) increase in twitch transdiaphragmatic pressure amounted to 2.9%.

The magnitude of effect might have been influenced by the concomitant small decrease in left EMG amplitude, and small increase in right EMG amplitude. Theoretical calculations based on the Pdi:Edi ratio predict a greater increase in twitch tension, had the EMG amplitudes remained constant throughout the study. In practice, there was no correlation between changes in electromyogram and twitch amplitudes, a finding confirmed by Levy and colleagues (1990), and the calculation

is, therefore, not reliable.

The results of Study 2 and of work by Levy et al (1990) are in agreement, with an increase of approximately 3% in bilateral twitch transdiaphragmatic pressure being found in both studies. They conflict with the report by Murciano and co-workers (1987), who found an increase of 23% in twitch tension with no change in left or right electromyogram amplitudes on aminophylline administration. Little information was provided by the latter group on alterations in lung volume and diaphragmatic configuration. In the study presented here, these parameters remained very similar during the two study periods. The different findings by Murciano et al (1987) might be due to an increase in diaphragmatic length, and thereby greater tension development, with aminophylline.

The study of quadriplegic patients (Chapter 5) allowed the acute effect of aminophylline on the diaphragm to be studied during an unchanging level of phrenic nerve stimulation and, in addition, without influence from the central nervous system. Because there were only two patients studied, with different parameters measured in each case, the results must be treated as anecdotal, and interpreted with caution.

Nevertheless, it is clear that diaphragmatic activation, as judged by bilateral diaphragmatic electromyograms, remained constant in both patients. In the first patient, in whom transdiaphragmatic pressure was also measured, diaphragmatic contractility on the third measurement day decreased from the first control pacing session to the second. This suggests that diaphragmatic fatigue occurred. Fatigue has been noted

previously in a number of patients, who like Patient 1 in this study, have been ventilated for several months, during which time the diaphragm undergoes atrophy (Oda et al 1981, Glenn et al 1984).

Following aminophylline in Patient 1, transdiaphragmatic pressure increased by 12.6%, but did not return to the control value, suggesting that aminophylline had attenuated, but not completely reversed, fatigue.

The second patient had electromyogram and tidal volume recordings. The latter increased, non-significantly, by an average of 8.3% after aminophylline, while there were no changes in electromyogram amplitudes. The patient had no evidence on history or examination of airway disease, so that it is likely that the small increase in tidal volume was due to enhanced diaphragmatic contraction.

Study 4 (Chapter 6) investigated the chronic effect of aminophylline on respiratory muscle and quadriceps femoris strength in normal subjects. A small (4.1%) but statistically significant increase in the maximal sniff was seen, suggesting that the drug genuinely increased inspiratory muscle strength. The corresponding changes in maximal inspiratory and expiratory mouth pressures were of similar magnitude, but were not significant. This might relate to the greater variability in day-to-day mouth pressure measurements when compared to sniff transdiaphragmatic pressure.

The lack of effect on maximal voluntary quadriceps contractions may be because the drug has a smaller action on the quadriceps femoris than on respiratory muscle. That this is

the case is supported also by the results of the first study.

A difference in response between muscles may be due to their functional, morphological, or cellular properties. The respiratory muscles are required to contract repeatedly throughout life while the quadriceps can rest without contracting for long periods. Gandevia and McKenzie (1987) stated that, in contrast to the inspiratory muscles, expiratory muscles and a limb muscle (the psoas) are unable to maintain 60% of maximal contractile force (Roussos et al 1979, Nickerson and Keens 1982). The production of fatigue in Chapter 3 shows that the endurnce capacity of the quadriceps femoris does not equal that reported for the inspiratory muscles. The results of several studies indicate that the percentage of Type II fibres are similar in the intercostal muscles, diaphragm, and limb musclea (Johnson et al 1973, Lieberman et al 1973, Wiles et al 1979, Gandevia & McKenzie 1987). Gandevia and McKenzie (1987) suggest that it is relative oxidative capacity rather than fibre-type proportions which might be responsible for dfiierences in endurance capaities between inspiratory and limb muscles.

The difference in the size of effect between Study 4 and other studies of voluntary respiratory muscle contractility, might be due to a greater response at low than at high stimulation frequencies, which are required for maximal efforts (Jones et al 1982). This is unlikely as the results of Study 4 concur with others on the sternomastoid, in which negligible changes in both low and high frequency force were reported with

aminophylline (Efthimiou et al 1986, Lewis et al 1986). The difference might relate to the length of treatment. No other work has studied the effect of chronic aminophylline administration on voluntary contractions in normal subjects.

In Study 5 (Chapter 7), maximal inspiratory mouth pressures in patients with COPD were greater on theophylline than on placebo (mean differences: 11% PImaxRV, 18% PImaxFRC). This level of improvement is more similar to that noted by Murciano and colleagues (1984, 1989), than has been detected by other studies of respiratory muscle contractility in COPD (Davidson et al 1984, Cooper et al 1985b, Foxworth et al 1988, Kongragunta et al 1988).

In the current study, there were no treatment differences in pulmonary function parameters, in particular, forced expiratory volume in one second, airway resistance or lung volumes, indicating that there were no substantial changes in diaphragmatic length at end-expiration or residual volume between the placebo and theophylline periods to explain the increased tension developed. No differences occurred in maximal expiratory mouth pressures or maximal voluntary quadriceps contraction, suggesting that the effect on skeletal muscle was restricted to the muscles working under a heavy load, ie. the inspiratory muscles.

The greater effect seen in Study 5, and in work by Murciano and co-workers (1984, 1989) than in other studies of patients with COPD may relate to the presence of other potentially fatiguing factors. Patients with hypoxia and/or hypercapnia (Murciano et al 1984, 1989) may be more susceptible to an

increase in inspiratory muscle strength than those with normal gases (Davidson et al 1984, Cooper et al 1985b, Foxworth et al 1988, Kongragunta et al 1988). Central nervous system stimulation may also be responsible, in part, for the findings in this patient population; hypoxic ventilatory drive is known to be increased by methylxanthines (Lakshminarayan et al 1978).

DIFFERENCE BETWEEN STUDY RESULTS

The results of the final study of this thesis appear to differ from the first four. It may not be valid to compare effects in patients with those in normal subjects. Patients with COPD appear to be more susceptible to fatigue, because of suboptimal inspiratory muscle function, than healthy subjects (Rochester 1981). Similarly, hypoxia, hypercapnia, hyperinflation, malnutrition, and the high level and long duration of inspiratory muscle contraction (tension-time index) may make the response of the respiratory muscles to methylxanthines different from that of normal subjects. This is supported by the study of patients with COPD (Study 5, Chapter 7). Patients had relatively poor inspiratory muscle function, as judged by their predicted values and the normal range (Wilson et al 1984a). The room for improvement in inspiratory muscle strength of the patients was considerably greater than that of the normal subjects in Study 4 (Chapter 6).

The findings in Study 3, (Chapter 5) also support the view that inspiratory muscle load and fatigue may be responsible for the difference in results. Patient 1 developed fatigue during phrenic nerve stimulation and this was partially reversed by

aminophylline. The size of effect was similar to that found in Study 5.

Another possible reason for the differences is that the threshold for the inotropic action of theophylline on skeletal muscle may be high, resulting in variable effects in individuals. Such variability was demonstrated in Study 5, in which only seven of the 10 patients had increased inspiratory mouth pressures with theophylline, and only two individuals showed increases in inspiratory mouth pressures at both lung volumes.

MAGNITUDE OF EFFECT

The results of the five studies indicate that dimethylxanthines do have a small inotropic effect on skeletal muscle contractility in normal man. The evidence for this is:

1. The difference in the fall in low frequency force (ie. at 20Hz) relative to maximum quadriceps force between theophylline and placebo of 1-2% (Chapter 3).

2. The increase in sniff transdiaphragmatic pressure with aminophylline of 4% (Chapter 6).

3. The increases in other strength measurements, while not statistically significant, were consistent and of the same order (1.5-4.8%. Chapter 6).

4. Although the results of Study 2 (Chapter 4) were not statistically significant, the concordance in the magnitude of effect for three of the four subjects, and with other work (Levy et al 1990), suggests that the mean recorded increase (2.9%) in twitch tension might be genuine.

A more substantial effect appears to occur in patients whose inspiratory muscles operate under a heavy workload, due to weakness, or because of airway obstruction, hyperinflation and hypoxia. The evidence supporting this is:

1. Patient 1 (Chapter 5) developed diaphragmatic fatigue after a short period of diaphragmatic pacing. Aminophylline partially reversed the fall in transdiaphragmatic pressure, increasing force by 12.6%.

2. The patients with COPD and hypoxia in Study 5 (Chapter 7) showed increases in inspiratory muscle strength of 11% (PImaxRV) and 18% (PImaxFRC).

Despite this benefit, patients had no concurrent improvement in visual analogue scales for general well-being or breathlessness, or in six minute walking distance. This is in keeping with others (Eaton et al 1982, Davidson et al 1984, Cooper et al 1985b). The lack of improvement in breathlessness and in exercise performance suggests that the day-to-day clinical benefit was limited. It may be that the inotropic effect of the drug is of most practical help when inspiratory muscles are severely stressed, during an acute exacerbation of airways obstruction, or a respiratory infection, when hypoxia, hyperinflation and elastic forces worsen.

COMPARISON OF EFFECTS ON SUBMAXIMAL AND MAXIMAL CONTRACTIONS

The inotropic action of theophylline extends to maximal as well as submaximal contractions. The evidence for this is:

1. In normal subjects, the maximal sniff increased significantly.

2. In patients, the paced submaximal transdiaphragmatic pressure was enhanced to a similar degree to maximal inspiratory mouth pressures.

As the two types of contraction were performed together in only two studies (Chapters 3 & 7), the exact magnitude of effect on each cannot be compared.

OVERALL STUDY CRITIQUE

The chief criticism of the studies in this thesis is that small numbers were involved. It is possible that statistically significant effects were not recognized. Nevertheless, the consistency in the magnitude of effect on skeletal muscle in the studies of normal subjects, and those of patients suggests that the size of effect has been correctly determined in these two groups. Therefore, in spite of the small numbers, the clinical significance can be judged from the data presented.

A major advantage was the unique design of the chronic studies. The design was double-blind, randomised and placebo-controlled. In addition, the protocols of Studies 1, 4 and 5 (Chapters 3, 6 & 7) entailed administration of aminophylline unblind for several days before both placebo and active drug ingestion. This thereby ensured that the two limbs of the study were identical, allowed tolerance to side-effects to develop, and obscured the identity of the succeeding agent. Studies of chronic theophylline administration by other workers have risked identification of the active agent by the patient, even when they have included double-blind randomisation and a placebo control. Without prior treatment it is easy for

subjects to recognise the active phase, and this may bias the results.

Conversely, the open and uncontrolled design of the acute aminophylline studies resulted in interpretation difficulties. The changes in compound muscle action potential amplitudes and the variability in individual results in Study 2 (Chapter 4) limit the usefulness of this study. Similarly, the order of sequential pacing sessions on Patient 1 (Study 3, Chapter 5) only partially assist in determining the relevance of the transdiaphragmatic pressure differences between the second control period and the aminophylline pacing period.

Despite these difficulties, the studies did help to answer the questions set out at the start: whether an inotropic effect occurs at therapeutic concentrations, the magnitude of effect in normal subjects and in patients, and whether maximal contractions are enhanced.

CONCLUSIONS

The conclusions that can be drawn from these studies are;

1. Theophylline has effects on respiratory and quadriceps femoris muscle contractility at therapeutic concentrations in man. This is clear from the results of Studies 1, 3, 4 & 6 (Chapters 3, 5, 6 & 7) and is supported by the similarity in results of Study 2 to those of the other studies in normal subjects.

2. The effects are more marked in muscles which are weak, under heavy loads, or at risk of fatigue. This is indicated by the fact that inotropic effects were seen in the study of

quadriceps fatigue (Study 1, Chapter 3) during the third frequency-force curves (FFCs), but not the first FFCs, both before and after the fatigue run. Further evidence is provided by the results of the studies on patients (Studies 3 & 5, Chapters 5 & 7), in whom more substantial effects were found than in normal subjects.

3. The inotropic effect of theophylline occurs at high frequencies of muscle stimulation as well as low frequencies. The difference in magnitude of effect at the two levels of muscle activation cannot be determined from this work, however. The occurrence of statistically significant drug effects both in studies of submaximal contraction (Studies 1 & 3, Chapters 3 & 5), and of maximal contractions (Studies 4 & 5, Chapters 6 & 7) support this contention.

4. The effects are small in normal man, greater in patients with hypoxia and/or at risk of diaphragmatic fatigue, but result in no more than a 20% increase in respiratory muscle contractility.

There are insufficient data to establish with certainty whether the inotropic action of theophylline on respiratory muscle would be of clinical benefit in patients with quadriplegia and phrenic nerve pacers, or in patients with COPD, who are at risk of diaphragmatic fatigue. An increase in inspiratory muscle contractility of 10-20% might be sufficient to prevent further deterioration in respiratory muscle function and progression to fatigue. The results would support a clinical trial in these small groups.

The risk-benefit ratio of theophylline in patients would need to be considered before use. A major side effect occurred in only two individuals, and no side effects were evident in the chronic studies following dose-titration. However, acute administration may result in serious events, limiting the practical usefulness of theophylline. A potentially less toxic agent, and one with a greater inotropic effect might be of considerably wider clinical benefit.

FURTHER STUDIES

Improvements could be made to the design of the studies in this thesis. An increase in cohort size of the studies would increase confidence in the findings. In particular, the two acute studies of twitch tension and the paced diaphragm would benefit from increased numbers. The practical difficulties of recruiting greater numbers are large, however. The studies entail considerable motivation on the part of the subject, invasive, often uncomfortable procedures, and the administration of a drug with unpleasant side effects. An alternative method of phrenic nerve stimulation, cervical magnetic stimulation, does not produce the pricking sensation in the neck associated with transcutaneous stimulation, and might encourage participation (Similowski et al 1989).

Another technical improvement on Study 2 (Chapter 4), would be to perform stimulation with subjects supine. Potential movements of the thorax and abdomen would be limited, so reducing the risk of movement of either phrenic nerve stimulating or electromyogram recording electrodes, and

minimising small changes in diaphragmatic length and configuration. Use of a brace might ensure constancy of position of the stimulating electrodes (Gandevia & McKenzie 1985). Alternatively, cervical magnetic stimulation might limit variable phrenic nerve stimulation (Similowski et al 1989).

A single-blind, placebo-controlled, randomised protocol on separate days might help to determine whether patients with new diaphragmatic pacers, and who are at risk of fatigue, can be paced for longer periods with dimethylxanthines. Such a protocol would require large numbers, to avoid bias by a treatment order effect.

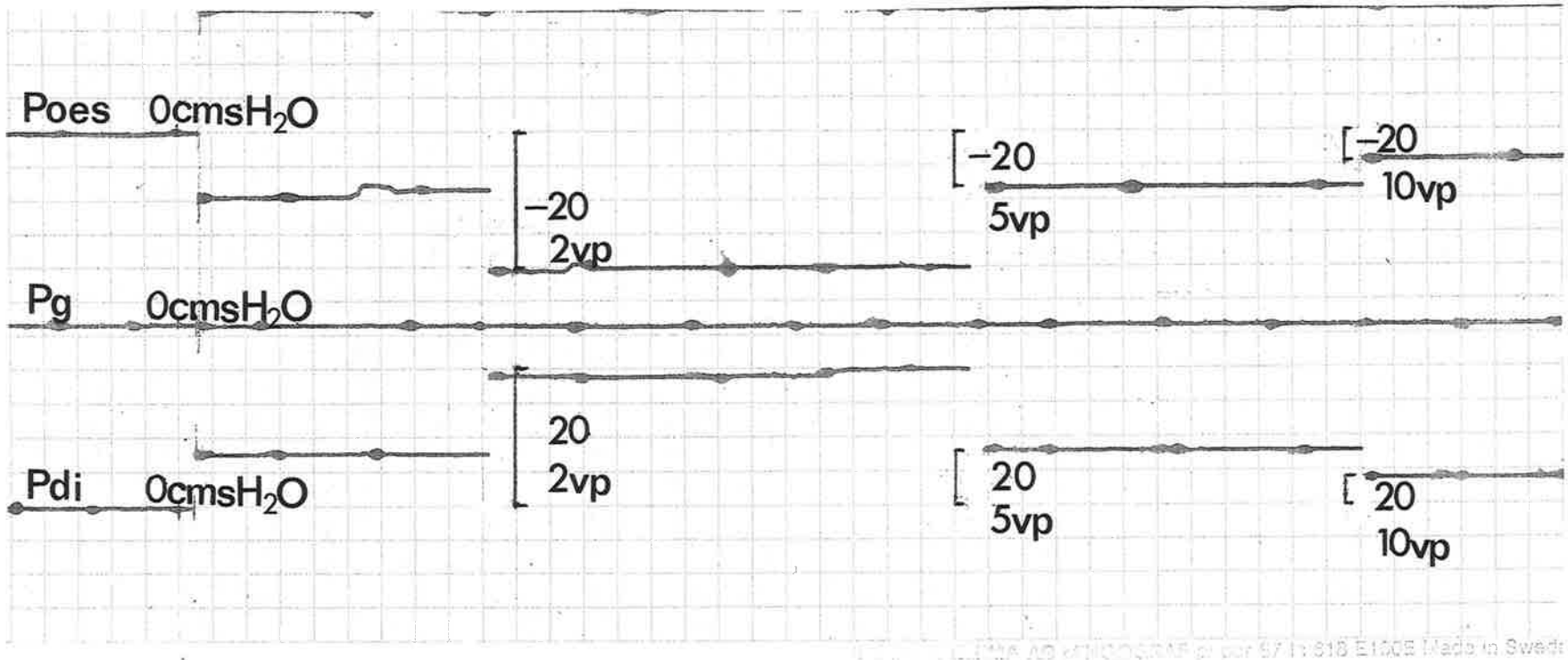
The study on the frequency-force relationship of the quadriceps femoris identified a fall in low frequency force from the first to the third frequency-force curves (FFCs) on placebo. This could be investigated by firstly increasing the interval between FFCs, and secondly, restudying the quadriceps an hour or more after the fatigue run, to determine whether the decrease in low frequency force during the third post-fatigue FFC was related to the gradual development of fatigue, or to the performance of frequent FFCs. The relevance of the small difference between placebo and theophylline low frequency force during the third FFCs, might thereby be clarified.

Other studies which investigate the benefit and extent of an inotropic effect of theophylline on respiratory muscle contractility in patients are also needed. In COPD, the effect of treatment over months, rather than weeks, on exacerbations, pulmonary function and exercise tolerance as well as on respiratory mouth pressures would provide data on the long-term

clinical benefit patients can expect from dimethylxanthines.

Asthmatics and patients with COPD with acute exacerbations or needing artificial ventilation, and patients with diaphragmatic weakness, caused by Guillain-Barre Syndrome or poliomyelitis could be investigated using the techniques utilised in this thesis. These are all groups at risk of respiratory muscle fatigue, which might be prevented by improvement in inspiratory muscle contractility.

APPENDIX


Poes 0cmsH2O
−20
2vp
−20
5vp
−20
10vp
Pg 0cmsH2O
20
2vp
Pdi 0cmsH2O
20
5vp
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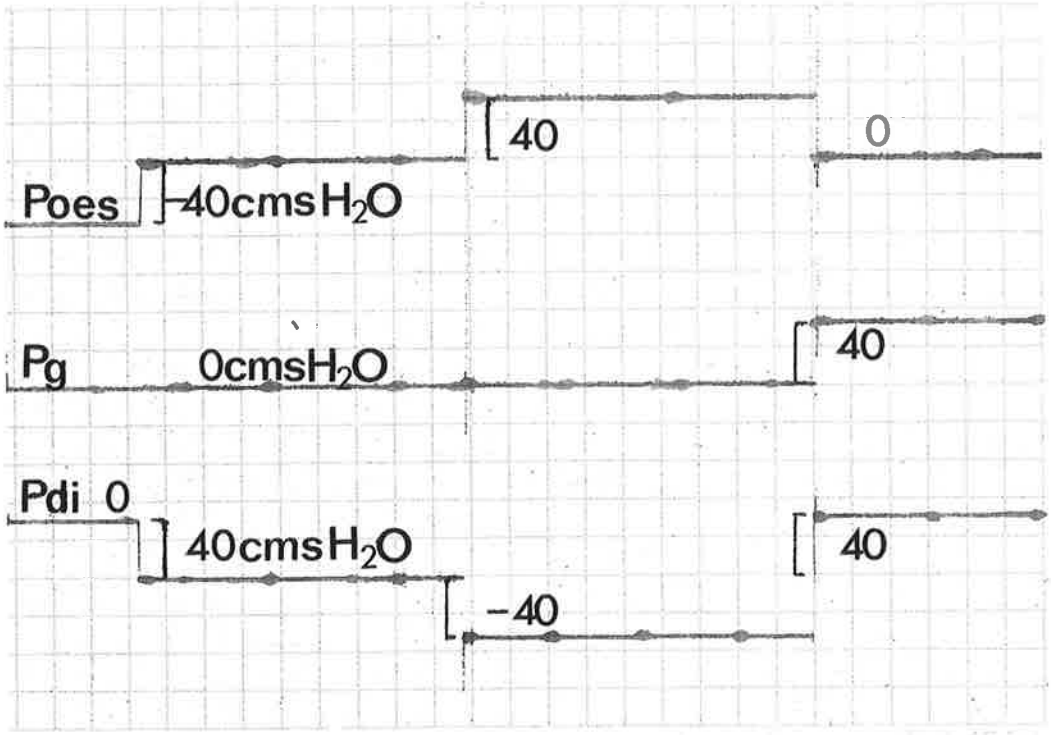
10vp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Poes
-40cmsH2O
Pg
0cmsH2O
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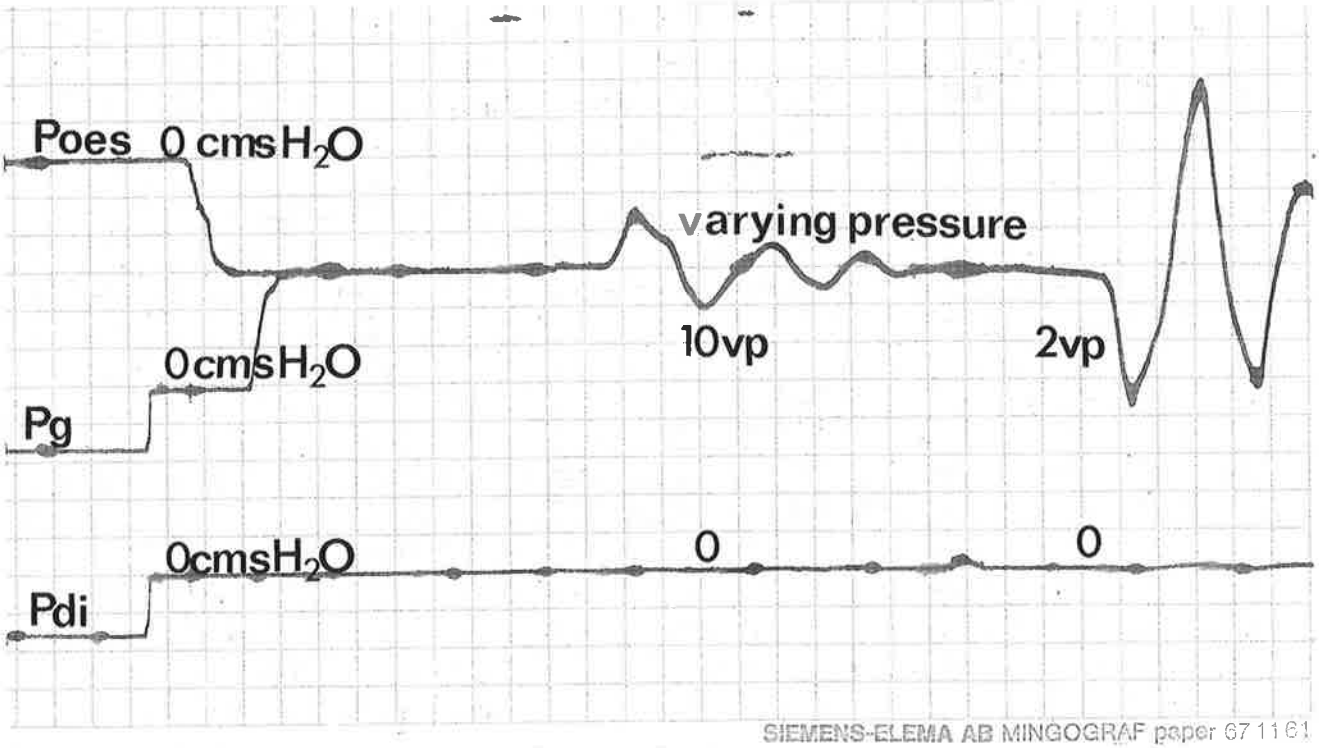
Pdi 0
40cmsH2O
40
-40


Poes 0 cmsH2O
varying pressure
10vp
2vp
0cmsH2O
Pg
0cmsH2O
0
0
Pdi
SIEMENS-ELEMA AB MINGOGRAF paper 671161

APPENDIX

An example of the calibration for the oesophageal pressure transducer and carrier amplifier (Poes), the gastric pressure transducer and carrier amplifier (Pg), and the electrical subtractor producing the transdiaphragmatic reading (Pdi=Pg-Poes). Pressures of 0, $\pm$20cmsH_2O and $\pm$40cmsH_2O were applied to each transducer at different gains (in terms of volts peak [vp]) on the Mingograf pen recorder (see Chapter 2, Section 2). In addition, a varying pressure was applied to both transducers simultaneously to demonstrate that the electrical subtractor operated under dynamic conditions.

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